Physiomorphological Response of *Lens culinaris* L. Medic. and *Vigna radiata* L. Wilczek to Pyridoxine Application

ABSTRACT

Thesis Submitted to the Aligarh Muslim University
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IN

Botany

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DEPARTMENT OF BOTANY
ALIGARH MUSLIM UNIVERSITY
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PHYSIOMORPHOLOGICAL RESPONSE OF LENS CULINARIS L. MEDIC. AND VIGNA RADIATA L. WILCZEK TO PYRIDOXINE APPLICATION

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Abstract of the thesis, submitted to the Aligarh Muslim University, Aligarh, India, for the degree of Doctor of Philosophy in BOTANY, 1986.

Six simple randomised field experiments - three each on lentil (Lens culinaris L. Medic.) var. T-36 (Experiments 1-3) and summer moong (Vigna radiata L. Wilczek) var. K-851 (Experiments 4-6)- were conducted at the University Farm and the Botanical Garden of the Aligarh Muslim University, Aligarh (India) from 1982 to 1984. The aim was to study the effect graded levels of pyridoxine applied by pre-sowing seed treatment and/or spray at flowering and fruit setting stages of these legumes on growth parameters, net assimilation rate (NAR), nitrate reductase activity (NRA), leaf NPK content, yield parameters and seed protein content. The data were mostly found significant and are summarised below.

Experiment 1 (Lentil): The effect of pre-sowing seed treatment for 12h with 0.0% (S_W), 0.1% (S_1), 0.2% (S_2), 0.3% (S_3), 0.4% (S_4) and 0.5% (S_5) aqueous pyridoxine solution was studied on

growth parameters, NRA and leaf NPK content at 60, 90 and 120d; NAR for 60-90d and 90-120d intervals and yield parameters and seed protein content at harvest during 1982-83. An unsoaked control (S_0) was also included in the scheme. Of these, S_3 proved optimum for almost all parameters, except most growth parameters at 60d that responded maximally to treatments containing lower pyridoxine concentrations. Treatments S_0 and S_W were at par in their effect on these parameters.

Experiment 2 (Lentil): The effect of spray at 90 or 110d of 0.0% i.e. $F_{W(90)}$ or $F_{W(110)}$, 0.1% i.e. $F_{1(90)}$ or $F_{1(110)}$, 0.2% i.e. $F_{2(90)}$ or $F_{2(110)}$, 0.3% i.e. $F_{3(90)}$ or $F_{3(110)}$, 0.4% i.e. $F_{4(90)}$ or $F_{4(110)}$ and 0.5% i.e. $F_{5(90)}$ or $F_{5(110)}$ aqueous pyridoxine solution was studied on growth parameters, NRA and leaf NPK content at 120d; NAR for 90-120d interval and yield parameters and seed protein content, at harvest during 1982-83. An unsprayed control, i.e. F_0 was also taken. Of these treatments, $F_{2(90)}$ proved optimum for almost all parameters, $F_{2(110)}$ being equally effective for yield parameters. Treatments F_0, $F_{W(90)}$ and $F_{W(110)}$ showed equal effect on these parameters.

Experiment 3 (Lentil): The combined effect of soaking the seeds for 12h in 0.0% (S_W), 0.2% (S_2), 0.3% (S_3) and 0.4% (S_4) and spray at 90d of 0.0% (F_W), 0.1% (F_1), 0.2% (F_2), and 0.3% (F_3) aqueous pyridoxine solution in sixteen combinations was studied on growth parameters, NRA and leaf NPK content at 60, 90 and 120d; NAR

for 60-90d and 90-120d intervals and yield parameters and seed protein content at harvest during 1983-84. Of these combinations, S_3+F_W proved optimum for most of the parameters, except growth parameters at 60d that responded maximally to S_2+F_W.

Experiment 4 (Summer moong): The effect of pre-sowing seed treatment for 4h with 0.0% (S_W), 0.1% (S_1), 0.2% (S_2), 0.3% (S_3), 0.4% (S_4) and 0.5% (S_5) aqueous pyridoxine solution was studied on growth parameters, NRA and leaf NPK content at 20, 30, 40 and 50d; NAR for 20-30d, 30-40d and 40-50d intervals and yield parameters and seed protein content at harvest in 1983. Of these, treatment S_3 proved optimum for most of the parameters, except plant length and root length at 20d and seed protein content that were maximum in S_1 and S_2 respectively.

Experiment 5 (Summer moong): The effect of spray at 35 or 45d of 0.0% i.e. $F_{W(35)}$ or $F_{W(45)}$, 0.025% i.e. $F_{1(35)}$ or $F_{1(45)}$, 0.05% i.e. $F_{2(35)}$ or $F_{2(45)}$, 0.1% i.e. $F_{3(35)}$ or $F_{3(45)}$ and 0.2% i.e. $F_{4(35)}$ or $F_{4(45)}$ aqueous pyridoxine solution was studied on growth parameters, NRA and leaf NPK content at 45 and 55d; NAR for 35-45d and 45-55d intervals and yield parameters and seed protein content at harvest in 1983. Treatment $F_{3(35)}$ proved optimum for all parameters studied, except root length at 55d (non-significant) and 1,000 seed weight that was optimum in $F_{1(45)}$. Treatment $F_{3(45)}$ was equally effective with $F_{3(45)}$ for pod length and seed number/pod. Treatments $F_{W(35)}$ and $F_{W(45)}$ proved at par in their effect on all parameters.

Experiment 6 (Summer moong): The combined effect of soaking the seeds for 4h in 0.0% (S_W) and 0.3% (S) and spray at 35 or 45d of 0.0% i.e. $F_{W(35)}$ or $F_{W(45)}$, 0.1% i.e. $F_{1(35)}$ or $F_{1(45)}$, 0.2% i.e. $F_{2(35)}$ or $F_{2(45)}$ and 0.3% i.e. $F_{3(35)}$ or $F_{3(45)}$ was studied on growth parameters, NRA and leaf NPK content at 45 and 55d; NAR for 35–45d and 45–55d intervals and yield parameters and seed protein content at harvest in 1984. Treatments $S+F_{W(35)}$ and $S+F_{W(45)}$ proved optimum for all parameters, except plant length and fresh weight at 45d and leaf number at both stages that were maximum in $S+F_{1(35)}$ and $S+F_{2(35)}$ respectively. Treatments $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ showed equal effect on all parameters.

Values of correlation coefficients were also calculated to understand the implications of the above findings. In all the six trials, growth characteristics, NAR, NRA, leaf NPK content and yield parameters showed correlation with seed yield. Similarly, NRA and leaf NPK content were found to be associated with seed protein content in both crops. This criterion could, therefore, be employed in predicting seed yield and quality of lentil and summer moong.

In conclusion, pre-sowing seed treatment with 0.3% pyridoxine proved optimum for both crops. However, among spray treatments, 0.2% and 0.1% applied at flower-initiation stage proved optimum for lentil and summer moong respectively. It was also noted that soaking in 0.3% pyridoxine showed superiority over

spray of the respective optimum levels for the two crops. Therefore, soaking of seeds of these crops in 0.3% pyridoxine solution may be exploited economically to augment the yield and seed quality of lentil and summer moong.

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ALIGARH (U.P.), INDIA

CERTIFICATE

This is to certify that the thesis entitled, "PHYSIOMORPHOLOGICAL RESPONSE OF *LENS CULINARIS* L. MEDIC. AND *VIGNA RADIATA* L. WILCZEK TO PYRIDOXINE APPLICATION" submitted in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in BOTANY is a faithful record of the bonafide research work carried out at the ALIGARH MUSLIM UNIVERSITY, ALIGARH by MR. SHAMIM AKHTAR ANSARI under my guidance and supervision and that no part of it has been submitted for any other degree or diploma.

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CHAPTER - 1

INTRODUCTION

INTRODUCTION

It is generally agreed that in response to the exigencies faced by him, early man started agriculture after recognising the nutritive value of different wild plants. It is, therefore, not surprising that side by side with cereals, leguminous crops were cultivated in the distant past. Of these, at least "masha" (urd, Phaseolus radiatus), "masura" (lentil, Lens culinaris) and "mudga" (moong, Vigna radiata) are known to be mentioned in early Aryan literature and date back to thousands of years (Achaya, 1985). The early Roman and Greek farmers have also been credited with the knowledge of the beneficial effect of rotating leguminous and nonleguminous crops (Bould, 1963; Burris, 1965).

With the advancement of science and technology, agriculture has emerged as an industry in most of the developed and some of the developing countries in recent years. As a rule, the success of an industry depends upon low energy investment, accompanied by high output, agriculture being no exception. Of the total energy invested in the agricultural sector of a developed country, like U.S.A., about a quarter is used for synthetic fertiliser production. Estimates show that there has been a several-fold increase in the input of nitrogenous fertilisers during the past few decades (Flaig, 1978) and there are indications that this trend will continue. The situation in

developing countries is even worse because of the general awakening among farmers coupled with constraints on fertiliser production. In India, for example, the existing nitrogen gap between production and consumption has been doubled within the last decade (Subba Rao, 1979), proving a heavy burden on the economy of the country.

Under these circumstances, judicious exploitation of the unique mechanism of dinitrogen fixation possessed by leguminous crops could help in more than one way. It would increase soil fertility, provide a cheap alternative for nitrogenous fertilisers and check environmental pollution. It is estimated that about 175 million metric tons (tonnes) of dinitrogen is fixed annually by various organisms (Burns and Hardy, 1975; p.54). The forage legumes contribute approximately 125-300kg/ha/year, and edible legumes 50-60kg/ha/year (Mishutin and Shilnikova, 1971).

Because of their high (20-40%) seed protein content, the role of these crops is even more important in India as the majority of the population is vegetarian and obtains most of its protein from the legumes. The "Green Revolution" has successfully met the increasing demand for cereals during the recent past when the country was threatened by famine due to the population explosion; but it eclipsed the need for increased legume cultivation. The consequences were inevitable. The compound

growth rate of legume production steadily declined annually by 0.45% from 1960-61 to 1977-78 and total production slumped from 13.04 million tonnes in 1975 to 8.4 million tonnes in 1980 (Anonymous, 1983).

Leguminous crops having been neglected by the plant breeders and agronomists, farmers continued to grow the available low yielding cultivars of these crops, without much information about their agronomy. Ironically, India is one of the major legume growing countries of the world as 24 million hectares of arable land (17% of the total cultivated area of the country) is occupied by these crops (Mehta, 1968). However, only about 12 million tonnes of legume grains or about 500kg/ha is produced in this country (Jeswani and Van Schaik, 1968; Mann and Singh, 1975) as against 3,494kg/ha produced by France, for example (Anonymous, 1984).

This low production of grain legumes was bound to have adverse effects on the population with wide-spread protein malnutrition prevailing all around. However, the gravity of the situation was belatedly realised by the national planners, and these crops have been given top priority for improvement in their genetic stock and agronomical practices. Keeping future needs in view, a target for the production of 14.5 million tonnes of improved legume seeds by the end of 1984-85 was set up (Anonymous, 1983). The farm scientists took up the challenge in real earnest.

The plant breeders evolved new high yielding varieties for various agro-climatic regions. Some of the newly evolved cultivars of moong (Vigna radiata) and urd (Phaseolus radiatus), being short-duration crops, could be grown during the non-conventional "zaid" (summer) season. These and other newly introduced cultivars were then subjected to intensive trials by agronomists for exploiting their full genetic potential by judicious application of fertilisers and proper management. At Aligarh, Samiullah and his associates have made significant contribution in the field within a short span of time (Akhtar and Samiullah, 1982; Samiullah et al., 1982, 1983, 1985; Akhtar et al., 1983, 1984; Akhtar, 1985). These studies have resulted in the establishment of optimum fertiliser doses and application schedules for some improved varieties of summer moong and lentil.

The present author visualised the problem from a different angle. He became particularly interested in the results of investigations carried out earlier in his laboratory on the effect of vitamin B_6 (pyridoxine) on the growth and productivity of barley and triticale (Afridi et al., 1979; Ahmad et al., 1981, 1982; Ashfaq et al., 1983). Preliminary studies revealed that, like cereals, seed treatment with pyridoxine enhanced the fresh weight and number of lateral roots in 10d old (2-leaf stage) urd seedlings grown in sand (Khan and Ansari, 1984) and root growth of lentil and summer moong in petridishes (unpublished).

These encouraging findings led the author to argue that such enhanced root growth would not only help the seedlings to get established quickly and bring about increased water and nutrient uptake (as in the case of cereals mentioned above), but also provide additional surface area for the rhizobia to produce more nodules. These would then be expected to fix more dinitrogen and thus enrich both the crop and the soil harbouring it. If successful, this technique could provide a viable farm practice particularly suited to the cultivation of grain legumes under dry-land conditions in which 15.3% (i.e. 90% of the area under legumes is unirrigated) of these crops are normally grown in India (Mann, 1968).

It was, therefore, decided to undertake six field experiments - three each on lentil and summer moong-to test the above hypothesis. These crops were selected in view of (i) diversity of genetic material; (ii) their fairly high seed protein and limiting amino acids (particularly methionine and tryptophan) content (Gupta, 1982; pp.297 & 301); (iii) well established agronomic practices under local conditions (Akhtar, 1985) and (iv) to keep the investigations fairly distributed throughout the year.

The aims and objects of these field trials were to study the effect of:

1. Pre-sowing seed treatment with graded aqueous pyridoxine solution

2. Foliar spray of graded aqueous pyridoxine solution applied at flower-initiation (90d in lentil and 35d in summer moong) or at fruit-initiation (110d in lentil and 45d in summer moong)

3. Combinations of seed soaking and foliar spray of pyridoxine solution

on growth, net assimilation rate, leaf nitrate reductase activity, leaf NPK content, seed yield and seed protein content of lentil (Lens culinaris L. Medic. var. T-36) and summer moong (Vigna radiata L. Wilczek var. K-851), both grown from rhizobium inoculated seeds.

CHAPTER-2

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

2.1 Vitamins

Vitamins are organic compounds which are required in trace amounts to maintain normal growth and proper development of organisms. However, they do not furnish energy and are not utilised as building blocks for the structure of the organism. These compounds act as co-enzymes in a number of enzyme systems and thus, take part in the regulation of metabolism. They were first recognised in animals and thereafter in plants because the latter are autotrophs and synthesise these substances, with the probable exception of vitamin D.

Generally speaking, three distinct periods in the history of vitamin research can be differentiated. The first period was characterised by the recognition of their existence. The second period was devoted mainly to the isolation of a number of vitamins in pure form and elucidation of their chemical structure which culminated in their laboratory synthesis. This period started about the middle of the 1920's when the first vitamin was obtained in the crystalline form. The final period was characterised by the recognition that these compounds, which were known for a long time to exert beneficial effects on the growth of micro-organisms and animals, were also necessary for the growth of higher green plants.

Funk in 1912 was the first to isolate an amine from rice husks and polishings that alleviated the symptoms of the disease "beriberi". He also proposed the generic term "vitamine" for it (Lehninger, 1982). Drumond (1920) dropped the terminal "e" of "vitamine" because many of the compounds of this group were not amines. The term "vitamin" coined by him was accepted by later workers. McCollum and Davis (1915) classified all vitamins into two groups, the fat soluble and the water soluble. The fat soluble vitamins included A, D, E and K, while water soluble vitamins covered B and C. B-vitamins further constituted a series of organic compounds designated as B_1 or thiamine, B_2 or riboflavin, nicotinic acid or niacin or pellegara preventive factor, B_6 or pyridoxine, biotin, B_{12} or cyanocobalamine (Lehninger, 1982). Wagner and Folkers in 1964 attempted to give a comprehensive definition of vitamins as:

(a) An organic compound

(b) A component of natural food but distinct from carbohydrate, fat or protein

(c) Present in normal food in extremely small concentrations

(d) Essential for normal health and growth

(e) When absent from the diet or not properly absorbed from the diet, causes specific deficiency symptoms

(f) Cannot be synthesised by the host and must, therefore, be obtained exclusively from the diet (distinction between vitamins and hormones).

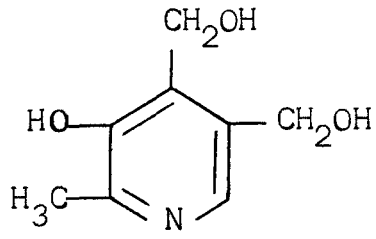
Later, Folkers in 1969 gave a modified definition of vitamins by taking into account the knowledge about the biosynthesis of nicotinic acid, vitamin-C and co-enzyme-Q. It read as "an organic substance of nutritional nature present in low concentration as a natural component of enzyme systems and catalyses required reactions and may be derived externally to the tissues or by intrinsic biosynthesis" (Morton, 1974).

The detailed mechanisms of the synthesis of most vitamins in plants are not known. Even their physiological roles in the plants are not as clearly understood as in animals. However, the role of B-vitamins in plants has been elucidated to some extent during the first half of the present century (Bonner and Bonner, 1948). It is noteworthy that the building units for the synthesis of vitamins are the same as those used by the plants for the synthesis of many other structural compounds.

2.2 Pyridoxine (syn. vitamin B_6, "rat acrodynia", factor of György, factor of elution, adermin)

György (1934) was the first to define and delineate vitamin B_6 as a distinct entity. It was isolated in crystalline

form from yeast by Kuhn and Wendt, from rice polishing by Keresztesy and Stevens, and by Ichiba and Michi (Wiarde, 1938). The term pyridoxine was coined by György and Eckhardt in 1938 and was soon adopted by the American Institute of Nutrition, the American Society of Biological Chemists and the Council of Pharmacy and Chemistry of the American Medical Association (Schöper, 1949). Harris and Folkers (1939), Harris et al. (1939) and Stiller et al. (1939) established that vitamin B_6 was a pyrimidine derivative, being 2-methyl-3-hydroxy-4,5-di(hydroxy-methyl)pyrimidine. Stiller et al. (1939) and Kuhn and Wendt in 1939 proposed the following chemical structure of pyridoxine (empirical formula: $C_8H_{11}O_3N$):



Pyridoxine

Crystals of vitamin B_6, as a free base, are colourless rods of varying size with rounded ends and appear to have a tendency to coalesce in rosette or fanshaped formations (György, 1938). It melts at 160°C (Keresztesy and Stevens, 1938) and is readily soluble in water, alcohol and acetone. The vitamin is

light sensitive and reacts easily with various acids to form salts. Vitamin B_6 is commercially available as its heat-stable, white hydrochloride salt, empirical formula: $C_8H_{11}O_3N.HCl$, melting point: 204-206°C (Keresztesy and Stevens, 1938) and is readily soluble in water (1g in 4.5ml) and alcohol (1g in 90ml).

Vitamin B_6 occurs in three active forms in living system, viz. pyridoxine, pyridoxal phosphate and pyridoxamine phosphate. It is extremely versatile in function and is involved in transformation of amino acids and in transferring their amino groups. It acts as co-enzyme for transaminases or amino transferases (Lehninger, 1982).

It may be added here that although considerable work has been done on the metabolic role of pyridoxine in animals, but the same cannot be said about plants. Even information about its distribution and biosynthesis in plants is meagre. In view of this and on account of its close relationship with other members of the B-vitamins, it is proposed to review the literature pertaining to the group as a whole in the following pages with particular emphasis on pyridoxine because of the specificity of the present research problem.

2.3 B-vitamins: Distribution, transport and excretion

B-vitamins are distributed in all plant parts. They are usually synthesised in mature green leaves which export them to

the growing organs displaying higher metabolic activities, like shoot apices, roots and reproductive organs. A part of these vitamins is also excreted through roots, which may provide suitable medium for the growth of microflora in the rhizosphere. Interaction of these organisms sometimes becomes beneficial for the growth and development of the plants.

2.3.1 Distribution

Conner and Straub (1941) found that wheat and corn contained higher concentration of thiamine than riboflavin. They proposed that thiamine content in wheat was presumably dependent on the variety, protein content and environmental conditions. In addition, wheat germ was found to have higher thiamine content than corn germ, but the riboflavin content of the two was approximately the same.

Burkholder and McVeigh (1942) and Burkholder (1943) estimated the B-vitamins in the germinating and dormant seeds of a number of crops, including barley, oats, corn, wheat, soybean, mungbean, pea etc. The concentration of riboflavin, niacin, biotin and pyridoxine was significantly increased during the germination of seeds. On the other hand, thiamine content in general did not change appreciably. However, thiamine content in pea embryos of germinating seeds was enhanced, while the content in cotyledons decreased.

Burkholder and McVeigh (1945a) observed that leaves of hemlock and winter buds of tulip, willow and forsythia possessed notably high thiamine content, while needles of hemlock and pine contained more riboflavin than the buds of these deciduous trees. On the other hand, pyridoxine was relatively abundant in buds of poplar, forsythia, tulip, apple and in the coniferous needles; niacin was high in alder, willow, poplar, birch, forsythia, and coniferous needles, and very high in maple. Similarly, biotin was high in apple, chestnut, alder, sassafras, linden and willow. However, total B-vitamins were high in hemlock and pine needles and in buds of tulip and forsythia. In another study, Burkholder and McVeigh (1945b) assayed thiamine, pyridoxine, niacin, biotin etc. in the germinating seeds of Pisum sativum, Phaseolus aureus and seven varieties of Soya max. On dry weight basis, niacin and riboflavin increased greatly during germination. Pyridoxine and biotin showed small gains, thiamine generally remained unchanged, but total B-vitamins decreased.

Hoffer et al. (1946) reported the distribution of thiamine in wheat seedlings. The seedlings, grown in complete darkness, showed no change in total thiamine content over a period of 18d of germination. However, total thiamine present in sprouts increased from the second to eighteenth day from 11 to 68% and decreased in germ end of the grain from 75 to 25% and in the brush end, from 14 to 7%. The results indicated that thiamine was translocated from kernel to developing sprouts.

Gustafson (1947) studied the distribution of thiamine and riboflavin in the tomato plant. The mature leaves and stem were found to possess the highest concentration of these vitamins. In case of mature leaves, all vitamins were accumulated in the leaf-blade. The roots contained much less thiamine than the middle parts of the plant. On the other hand, riboflavin in roots was as much as in the middle part of the plant, but less than that present in the apex of the plant. With the increasing age or size of the plant, vitamins continued to increase upto 4 to 5 months. It was also observed that the mature leaves contained more vitamins than the ripe fruits.

Wilson (1947) investigated the distribution pattern of vitamins during the development of 12 lines of cucurbit fruits. There appeared a trend in all lines of Cucurbitaceae, characterised by high concentrations of B-vitamins in young ovaries and marked reduction in vitamin levels during later stages of the development. In addition, relatively high concentrations of vitamins were maintained in the majority of lines till the flowering time, and the amount of vitamins was reduced largely between flowering and maturity. The concentration of niacin was high compared with that of thiamine or riboflavin. At maturity, the concentration of thiamine regularly increased in placental region, while concentration of this vitamin and/or niacin increased occasionally in the inner wall of the fruit and a decrease was noted in the outer wall. On the basis of these observations, it was inferred

that the increased concentration of thiamine was correlated with seed production and development. In general, the vitamin contents were correlated with the age of fruit rather than their size.

Withner (1949) studied the B-vitamin changes during the development of cucurbit and tomato leaves. Ten B-vitamins were assayed in stem tip leaves, young enlarging leaves and mature leaves in each of three tomato varieties (Red Currant, Red Cherry and Stokesdale) and of three inbred cucurbit lines (TA, O and BT). Of the vitamins studied, thiamine, niacin and biotin occurred in greatest amounts in the top leaves, while the concentration of pyridoxine and riboflavin was often higher in the mature leaves.

These studies clearly indicated that B-vitamins are universally distributed in all green plants. However, the concentration of each vitamin of this group varies with the organ, age and type of the plants.

2.3.2 Transport

Bonner (1942) extensively studied the translocation of thiamine in the tomato plant by using girdling technique in which phloem was removed. It was found that thiamine was accumulated above the girdle in stems and there was a marked depletion of thiamine below the girdle and roots. After the sixth day of girdling, the total amount of thiamine in 1cm of stem above the girdle was 2.8 and 3.5 times as high as the concentration in the

corresponding portion of the stem of the control plant (without girdle) and 1cm below the girdle respectively. In another experiment, stems were girdled between mature leaves and the young rapidly growing leaves and the other leaves of intermediate age were removed. In this case, thiamine was accumulated below the girdle, and its concentration was nearly twice that above the girdle. These studies showed that thiamine was transported from the mature leaves bidirectionally through the phloem. Girdling of petiole further supported the inference that mature leaves synthesised and transported the thiamine to the other vegetative parts of the tomato plant, as this vitamin was found to be accumulated more in the lamilar side of the petiole of the mature leaves than in that of the younger leaves. Further, there was no depletion of thiamine in stem towards girdling of yeung petioles. Similarly, he also evaluated the relative contribution of young and mature leaves regarding the accumulation of thiamine above the girdle. It was found that plants without mature leaves, did not accumulate thiamine above the girdle, suggesting that mature leaves exported thiamine to the roots. As for root-shoot competition to derive thiamine factor, decapitated plants were used, and it was found that these plants contained high thiamine content in roots compared with the normal plants. It revealed that there was a competition between root and shoots to derive this vitamin from mature leaves.

Pyridoxine, like thiamine, was produced in the leaves of tomato and was exported from the leaves to roots. Rapid accumulation of pyridoxine occurred in the stem above the girdle (Bonner and Dorland, 1943a). On the other hand, riboflavin did not accumulate to any extent in the regions of the stem above the girdle and would hence appear not to be transported towards the roots (Bonner and Dorland, 1943b).

In the light of these studies, Bonner and Bonner (1948) concluded that thiamine and pyridoxine formed in the leaves were required and derived by the roots as growth substances because roots were unable to synthesise these vitamins. Conversely, riboflavin, which seemed to be synthesised by roots, appeared to be synthesised by other tissues also and was not translocated. The synthesis and transport of other B-vitamins in higher green plants have not been well established and studied.

2.3.3 Excretion

It is well established that plant excretes a number of inorganic and organic substances from leaf, stem and root. The exudates discharged from the roots are mostly organic substances, e.g. phytohormones, amino acids and vitamins. The concentration and nature of these organic compounds differ from species to species. These compounds, particularly vitamins, provide good medium for the growth and development of the microflora in the rhizosphere.

West (1939) analysed the root exudates of Bison and Novelty flax, grown in petridishes for four days and thereafter transplanted in test tubes containing nutrient solution. After one, two and three weeks of growth, the nutrient solution from five test tubes, containing one plant per tube, was concentrated and assayed for thiamine and biotin contents. The thiamine was excreted 0.23 and 0.64 µg from five Bison and 0.24 and 0.64 µg from Novelty seedlings, after one and two weeks of growth respectively. As for biotin, it was excreted in quantities, 0.06, 0.25 and 0.21 µg from Bison and 0.08, 0.20 and 0.20 µg from Novelty seedlings, after one, two and three weeks of the growth respectively. He also isolated one hundred different representative bacteria from each of the rhizopsheres of Bison and Novelty flax, after three weeks' growth in uniform soil under green-house conditions, and from the rhizospheres of two tobacco varieties grown in the field condition. It was noted that microflora population varied in the rhizosphere of these plants. On the basis of these observation, it was suggested that the plants might have excreted the necessary growth factors for their nutrition.

Epanchinov (1973) observed the excretion of B-vitamins as influenced by mineral fertilisers, and their subsequent effect on the growth of maize, at 3-4 leaf stage, under field conditions. It was observed that dressing of fertilisers caused the accumulation of nicotinic acid, biotin and pyridoxine in soil

around the root zone of maize. He concluded that the mineral fertilisers produced a positive effect on maize and favoured the accumulation of these vitamins in the soils. According to him, these vitamins in turn intensified the growth of maize seedlings by facilitating the entry of nutrients into them.

2.4 B-vitamins as growth factor

Considerable research has been done to investigate the role of these vitamins in plants. Most of the studies have been confined to excised organ culture particularly of roots, as green plants are able to synthesise B-vitamins. The data revealed that these vitamins were necessary for normal growth of plant organs. However, little work has been done with entire plants. Moreover, the response of crops to the application of these vitamins has also been observed by very few workers who administered B-vitamins either to seeds or to leaves. These researches regarding the effect of B-vitamins on plants, though varied, are still far from complete and are reviewed below.

2.4.1 Studies with excised organs

Bonner (1937) observed the effect of yeast extract on the growth of excised pea roots. Initially, yeast extract exerted an inhibitory effect. However, yeast extract became essential for the growth when such excised roots were sub-cultured several times

by removing 10mm tips and placing them in fresh culture medium. Pea roots ceased to grow completely after three transfers in the absence of yeast extract. Presence of 0.01% yeast extract supported growth of excised roots in several passages with an average rate of 6 to 9mm/root/day. Later, he replaced yeast extract in the culture medium by vitamin B_1 and obtained normal growth of excised roots. On the basis of these observations, he inferred that yeast extract might have contained vitamin B_1 which was an essential growth factor for the excised roots. Subsequently, Bonner and Addicott (1937) reported that yeast extract furnished more substances than vitamin B_1 for maintaining normal growth and development of excised roots of pea, as vitamin B_1 alone in place of yeast extract was unable to support the continuous optimal growth of the roots in later passages. However, a mixture of amino acids along with vitamin B_1 yielded satisfactory results. It was also revealed that vitamin B_1 was required in traces. These observations showed that vitamin B_1 might have been supplied to roots by other parts of the intact plants and thus, they regarded it to be a phytohormone.

Robbins and Bartley (1937) applied equimolar mixture of thiamine fragments, namely thiazole and pyrimidine and maintained luxuriant growth of excised tomato roots in the absence of thiamine. However, response of different tomato strains was varied. In some strains, thiazole replaced thiamine without adversely affecting the growth of the roots. It indicated that

the other portion of thiamine, i.e. pyrimidine, was synthesised by excised roots of such tomato strains.

White (1937a) fractionated yeast extract for the study of its beneficial effect on excised roots of tomato. It was observed that only 18% of yeast extract, soluble in 85% ethanol, was responsible for the growth of roots. This material was again extracted with absolute ethanol and separated into two fractions; both fractions were found essential for growth. The material insoluble in absolute ethanol contained considerable amount of amino acids. Subsequently, White (1937b) reported the chemical nature of absolute ethanol soluble fraction of the yeast extract which was found to be vitamin B_1 and was considered as indispensable factor in the nutrition of excised tomato roots. Moreover, amino acids seemed to be accessary growth factors.

Bonner (1938) reported that pea root tips had considerable "reserve" vitamin B_1 and did not require its addition to the culture medium for their optimal growth until the root had grown 60mm or more. Excised roots were also able to utilise an equimolar mixture of thiazole and pyrimidine successfully. It led him to conclude that these molecules were synthesised <u>in vivo</u> to produce the vitamin molecule itself. It was also observed that excised roots were able to metabolise various derivatives of thiazole. However, thiazole possessing only hydroxyl group, showed activity as the growth factor.

Addicott (1939) found that vitamin B_1 enhanced the meristematic activity in the tips of excised pea roots. However, its effect was different to that of auxin, as the latter primarily promoted cell elongation, and in these studies, cell elongation, differentiation and maturation was normal in the absence of this vitamin.

Addicott and Devirian (1939) investigated another growth factor from the yeast extract which was essential, in addition to vitamin B_1, for the growth of excised pea roots. This second growth factor was not among the amino acids. They assumed it to be nicotinic acid as this vitamin in combination with vitamin B_1 supported growth of excised pea roots indefinitely which surpassed the growth, receiving vitamin B_1 only.

Bonner and Devirian (1939) cultured a number of excised roots of various plants in various nutrient media. Isolated pea roots grew for an unlimited period in a medium containing vitamin B_1, nicotinic acid, mineral salts and sucrose at the rate of 70-85mm/week. This growth rate remained unaltered even though various other chemical compounds, including vitamin B_2, B_6, C, E, K, adenine, theelin, β-alanine, pantothenic acid and numerous amino acids, were added. The rate of growth of isolated radish roots was 15mm/week when they were grown through 15 passages in the presence of vitamin B_1 and nicotinic acid. These vitamins were found indispensable to maintain normal growth of the roots

of these plants. The addition of other chemical compounds listed above, was found ineffective. Similarly, excised flax roots responded only to vitamin B_1 and grew at the rate of 150mm/week. On the other hand, excised roots of tomato showed a growth rate of 40mm/week in the presence of vitamin B_1 and B_6. This rate was further enhanced upto 60mm/week on supplementing the nutrient medium with nicotinic acid. These studies revealed that excised roots of various plants required different growth factors in the nutrient medium.

Robbins and Schmidt (1939a,b) found that addition of light brown sugar in nutrient medium was more beneficial for the growth of excised tomato roots than pure cane sugar. The root growth decreased when light brown sugar was replaced by its ash (treated with hydrochloric acid) or pure cane sugar containing nicotinic acid, nicotinamide, thiamine and amino acids. However, addition of pyridoxine (vitamin B_6) with pure cane sugar in the nutrient medium, promoted the growth of tomato roots. Addition of pyridoxine also induced the development of hooks and curls, indicating that it caused the elongation of cells. These observations showed that light brown sugar contained some root growth factor.

Bonner (1940) investigated root growth factor requirements of several plants <u>in vitro</u>. Excised roots of alfalfa, clover and cotton needed vitamin B_1 and nicotinic acid

for their optimum growth, which did not improve further on the addition of vitamin B_6 in the nutrient medium. On the other hand, roots of Datura stromonium and sunflower showed profuse growth in the presence of vitamin B_1, B_6 and nicotinic acid. Similarly, isolated roots of carrot required vitamin B_1 and B_6 but the addition of nicotinic acid was of no use. In case of five different strains of tomato, vitamin B_1 and B_6 proved beneficial for root growth which was further promoted by the inclusion of nicotinic acid into the medium. At the same time, excised roots of clover and flax were found to synthesise vitamin B_1 in small amounts. Therefore, they maintained sub-optimal growth even in the absence of this vitamin.

White (1940) observed the effect of vitamin B_6, nicotinic acid and pyridine in the presence of sufficient thiamine in nutrient medium, on the growth of excised roots of two tomato strains. Surprisingly, these strains of tomato did not significantly respond to vitamin B_6, nicotinic acid and pyridine. Therefore, he concluded that these strains of tomato did contain adequate amount of these substances to support the root growth under experimental condition.

Addicott (1941) studied the deficiency symptoms of hormone, vitamin B_1 and nicotinic acid on excised tomato roots. It was found that deficiency of either of these substances adversely affected root growth which sometimes ceased completely. It was

accompanied by a decrease in the length of meristem and cell divisions. Length of cell was also decreased at maturity. Deficiency of vitamin B_1 resulted in smaller diameter of root meristem and roots deficient in nicotinic acid showed a reduction in cell diameter as well as in the number of vertical rows of root cells. However, deficiency of vitamin B_1 retarded root growth more rapidly than that of nicotinic acid. Moreover, roots which were deficient in both the vitamins developed irregular thickenings at maturity.

Day (1941) successfully cultivated excised tomato roots on modified Pfeffer's solution containing, agar-sucrose. Thiamine, pyridoxine, nicotinamide, neopeptone, glutamic acid and glycine were added in various combinations to this nutrient medium. In the presence of thiamine, roots grew about 2mm daily which was further increased upto 5–6mm or even more on addition of pyridoxine. However, supplementing this medium with nicotinamide did not alter the growth rate appreciably. It was noted that tomato roots could be cultivated through 20 passages (more than 200d), on the agar medium enriched with thiamine and pyridoxine, without change in the growth rate. Further, presence of thiamine and pyridoxine in the agar medium induced the development of hooks and curls in the root. It was observed that pyridoxine could be replaced by glutamic acid or glycine.

Robbins (1941) studied the effect of various vitamins on root growth of two inbred tomato lines, viz. Red Current and Johannesfeur , and their heteroiic F_1 generation *in vitro*. The roots were supplied with thiamine, thiamine and pyridoxine or thiamine, pyridoxine and nicotinamide in the culture medium. These lines responded differentially to the vitamin application. The roots of F_1 produced more dry matter and showed better growth than either parent . However, the root growth of Red Current was luxurient in the presence of thiazole which surpassed even the F_1 in one of the passages. It also responded more than Johannesfeur to nicotinamide. The growth of Red Current became equal to that of hybrid F_1 if it was cultivated on a nutrient medium containing all the three vitamins. It seemed that the three lines of tomato had got different ability for thiamine, pyridoxine or nicotinamide synthesis which was presumably responsible for the variable effect of the application of these vitamins on their root growth. On this basis, the maximum growth of hybrid F_1 roots was interpreted as they had inherited characteristics of synthesising adequate amount of these vitamins cumulatively. On the other hand, better root growth of the hybrid in the medium containing all three vitamins was explained by assuming the higher synthesis of an unidentified growth factor in these roots.

Robbins (1942) investigated the effect of twelve analogues of pyridoxine on the growth of excised tomato roots in a medium supplemented with thiamine. Of these, nine analogues

were found ineffective for root growth. Acetylation and substitution of ethyl for the methyl group in the second position of pyridine ring did not alter the beneficial activity of the vitamin. These studies, thus, indicated that pyridoxine had high degree of specificity for the growth of excised tomato roots.

Day (1943) found that excised tomato roots seldom grew after two transfers if agar nutrient medium lacked thiamine, pyridoxine, nicotinamide and amino acids. However, presence of thiamine only in the medium supported the growth at the rate of 1.77mm/d for an indefinite period. This growth rate was further accelerated to 5.2mm/d on inclusion of pyridoxine accompanied with the development of hooks and curls. The addition of nicotinamide did not affect the growth rate, while neopeptone decreased it.

Hilderbrandt et al. (1946) worked out nutrient media for culturing tobacco and sunflower tissues. It was found that only sunflower tissue required pyridoxine in the basal medium for enhanced growth.

Almestrand (1950) worked out the growth factor requirement of excised wheat roots. He included three vitamins, viz. niacin, thiamine and pyridoxine, in the study. Of these, pyridoxine alone was found to be needed for the growth of wheat roots. It accelerated meristematic cell divisions. Addition of 0.5 to 1.0mg pyridoxine/l of culture medium at 27-28^{o}C proved optimum for wheat root growth.

Whaley et al. (1950) tested nutritional value of thiamine, niacin and pyridoxine for the growth of excised tomato roots. White's solution, enriched with sucrose and glycine, was used as culture medium. The data revealed that these roots required thiamine and niacin or pyridoxine for their optimal growth. Moreover, thiamine acted synergistically both with niacin and pyridoxine.

Almestrand (1951) observed the effect of pyridoxine and its two derivatives on the root growth of several strains of wheat, barley and oats as well as one of rye. No strain of barley, oats and rye responded to pyridoxine treatment, while different strains of wheat showed variable response to this vitamin. For instance, pyridoxine did not affect the root growth of Ergo II, Virtus and Pondus markedly but Eroica root growth was considerably enhanced by pyridoxine application. He also noted that pyridoxine application enhanced the uptake of glucose, phosphate and nitrate in roots of the pyridoxine-sensitive variety, i.e. Eroica. The uptake of these substances corresponded to the dose of applied pyridoxine. Moreover, derivatives of pyridoxine, i.e. pyridoxal and pyridoxamine, had similar beneficial effect to that of the mother compound.

Lee and Whaley (1953) cultured tomato roots for four weeks in two media: (a) without the supplementation of vitamins (b) supplemented with thiamine, niacin and pyridoxine individually

or in combinations. The data were collected at weekly intervals. Growth in all media was similar in the first week. But during fourth week, the growth in all culture media declined. Therefore, the best period for investigation was considered to be second to third week. Optimum root growth was recorded in the media containing thiamine alone or in combination with either of the other two vitamins, suggesting an inter-relationship among these vitamins to support normal root growth.

Similarly, tomato roots were cultured by Boll (1954) with the supplementation of vitamins to the nutrient medium. These roots required thiamine, pyridoxine and niacin for optimal growth. However, the growth could also be achieved if the medium contained only thiamine and pyridoxine. Moreover, pyridoxine and niacin in the medium were replaceable by pyridoxal or pyridoxamine and niacinamide respectively. It was also observed that niacinamide proved more active in comparison with niacin. Various derivatives of pyridoxine showed the order of effectiveness as pyridoxal > pyridoxine > pyridoxamine. Surprisingly, glycine was found to replace pyridoxine partly. This replacement became more effective in the presence of niacin. It was revealed that glycine and pyridoxine exerted similar morphological change in tomato roots. However, glycine affected initiation of lateral roots independently. These observations clearly established that a balancedsupply of the growth substances in the basal medium determined the morphology of root.

Crescimanno (1954) conducted experiments for two years to study root growth promotion in vine root stock cuttings. It was observed that vitamin B_1 affected root differentiation. He reported that distilled water containing vitamin B_1 accelerated rooting in vine cuttings *in vitro*.

Fujiwara and Ojima (1954) studied the effect of thiamine, pyridoxine and niacin on excised root tips of rice and wheat. The roots of these two plants responded positively to thiamine or pyridoxine application. However, in case of wheat, pyridoxine gave the best results. Moreover, application of vitamins did not show any effect on roots attached with their scutella. These roots grew much longer than excised roots.

Fries (1955a) compared the biosynthetic capabilities of decotylised pea seedlings grown in the dark with those of excised roots of the same plant. The decotylised pea seedlings required a mixture of water soluble vitamins and various amino acids in sucrose-mineral-salt medium for their optimum growth. Excised roots, on the other hand, remained unaffected by the application of these substances and these roots attained a length of 150mm even in the absence of the vitamins from which he inferred that excised roots had adequate reserve of vitamins. However, the roots ceased to grow after one or two transfers but the growth was again maintained on supplying thiamine and niacin. In case of pea seedlings, the growth diminished very soon in a medium lacking the

vitamins and main root did not grow beyond 100mm. The growth of seedling was further maintained by inclusion of thiamine and pyridoxine. However, niacin showed either poor or no effect. These studies showed that shoots failed to produce vitamins in the dark and consumed the vitamin reserves of the hypocotyl and young roots for their growth.

Fries (1955b) worked out the doses of thiamine and pyridoxine which could support the growth of decotylised pea seedlings in the dark on an agar-nutrient medium containing niacinamide and various amino acids. 10 µg of thiamine and 100 µg of pyridoxine/l were required to maintain normal growth of the seedlings. However, the pattern of growth and development of the seedlings was regulated independently by thiamine, pyridoxine and niacin. It was also observed that even in light the added thiamine alone controlled the growth rate considerably, suggesting that the synthesis of thiamine in light could not keep pace with the optimum requirement of the seedlings.

Willemot and Boll (1962) noted changes in the response of excised tomato roots to pyridoxine application after culturing these roots through several passages for one year. They isolated a subclone of this clone which did not show the requirement of pyridoxine in the solution for its growth. It was presumed that excised roots might have got adapted to grow in the absence of this vitamin and named this phenomenon as "Gautheret's accutumance

or anergie". However, the event of mutation had been ruled out as it needed to occur in many cells of meristematic zone simultaneously.

Das and Das (1966) observed the growth of excised pea roots as influenced by thiamine and pyridoxine treatment. They reported that these two vitamins showed similar growth promoting activity. However, the optimum dose of thiamine and pyridoxine differed, being 0.1 and 0.01ppm respectively. It was pointed out that vitamins might become more effective in presence of mineral salts.

Gašparíková (1968) observed the effect of auxin (IAA) and vitamin B_1 on the growth of excised tomato roots and found that IAA at a concentration of 10^{-7}M antagonised the growth promoting effect of thiamine on the excised roots. In general, thiamine alone at a concentration of $3x10^{-7}$M stimulated the root growth.

Galzy (1969) studied the effect of temperature on the efficiency of B-vitamins for root and shoot growth of cuttings of Vitis rupestris. It was found that B-vitamins at 20°C improved root growth only whereas at 35°C both root and shoot growth were stimulated.

Kumar et al. (1972) found that addition of vitamins (thiamine, nicotinic acid, inositol, pantothenate and choline

chloride) to nutrient medium separately or in combination stimulated tissue growth and chlorophyll formation in Dolichos lablab. The optimum doses of these vitamins were 1 and 2mg/l respectively.

Kozin and Kravtsov (1973) supplemented the nutrient medium with pyridoxine and cultured embryos of pear and apple. These embryos were isolated from seeds of different ripeness. They observed much higher germination, differentiation of embryo into seedling and accumulation of chlorophyll as a result of pyridoxine treatment. However, embryos from unripe seeds showed more pronounced response to pyridoxine than those of ripe seeds.

Ohira et al. (1976) reported the effect of thiamine on cells of various plants in suspension culture. Thiamine was found to be essential for the growth of soybean, tobacco and rice cells. The critical level of the vitamin required by these plant cells was about 0.6, 0.5 and 0.2 µg/g dry weight respectively. Further, soybean cells grew satisfactorily when equimolar mixture of pyrimidine and thiazole (thiamine precursors) was added in the suspension medium. However, none of these substances alone could replace thiamine. Experimenting with Ruta and peanut cells, it was observed that these cells did not require thiamine for their growth through ten passages; but Ruta cells growing with thiamine increased their thiamine content from 0.5-0.6 to 3.5 µg/g dry weight in the presence of light.

2.4.2 Studies with intact plants in tube, pot and field

The effect of B-vitamins on intact plants has been comparatively little studied. Of these, information with regard to pyridoxine is very meagre. In all these studies, the vitamins were applied either for soaking the seeds before sowing or to roots with the nutrient solution. The available literature on this aspects is presented below.

Bonner and Greene (1938) reported an increase in vitamin B_1 content of leaves and root tips of pea plants when kept in the light. Conversely, the plants growing in the dark had lower vitamin B_1 content in all plant parts. The root and shoot growth of plants grown in the dark was enhanced when vitamin B_1 was supplied to them through roots in small amount. It indicated that leaves synthesised vitamin B_1 in the presence of light and transported it to growing root tips, while dark grown plants needed exogenous supply of this B-vitamin for normal growth. They also observed the vitamin requirement of slow and fast growing plants under green-house conditions. The slow growing species of plants, e.g. Aleurites, Bougainvillea, Arbutus, Eucalyptus and Camellea, responded to thiamine, supplied with Hoagland's solution in sand culture. After two months, it was recorded that the shoot length became doubled compared to the respective control. Similarly, the root systems of the treated plants also exhibited more luxurient growth than those of control plants. However, fast

growing species of annual plants, like pea, radish and tomato showed no response to the vitamin treatment. They also noted that organic manure contained considerable amount of vitamin B_1 which might exert beneficial effect on plant development. Moreover, the vitamin B_1 content of soil might be derived from plant debris and the activity of microflora.

In a later study, Bonner and Greene (1939) observed the effect of vitamin B_1 on various plants under greenhouse and field conditions. In general, addition of 0.01mg vitamin B_1 of nutrient solution enhanced the rate of dry matter accumulation in plants in sand culture as well in soil. In sand culture, carob seedlings, receiving vitamin B_1 with nutrient solution on alternate days for a year, showed higher growth rate than the control. This effect of vitamin B_1 was not temporary. Cosmos, Poa, Brassica etc., exhibiting low vitamin B_1 content, responded positively to the vitamin treatment, while tomato and Pisum sativum with high leaf vitamin B_1 contents were non-responsive. They also analysed the vitamin B_1 concentration in leaves of responsive and non-responsive varieties of Pisum sativum and found that non-responsive varieties possessed more than double concentration of B_1 than the responsive one. On the basis of these observations, they suggested that leaf vitamin B_1 content might be taken as a criterion to decide whether or not to treat the plants with vitamin B_1 for better performance.

Arnon (1940) tried vitamin B_1 on tomato, lettuce, cosmos, mustard and cocklebur in water culture. The vitamin was added in concentrations of 0.1 and 0.05mg/l of nutrient solution. He found that these plants did not respond to the application of the vitamin. Therefore, he advanced the view that seeds of green plants possessed all essential substance (including B-vitamins) in adequate quantities for early growth of seedlings. Similarly, Hamner (1940) reported that vitamin B_1 did not influence the growth of cabbage, mustard, dahlia, radish and zinnia in sand culture. The same effect was noted even when cocklebur and cosmos received the vitamin twice and thrice a week respectively. Moreover, the effect of the vitamin was non-significant under various conditions, including long and short photoperiod as well as high and low supply of nitrogen nutrition. The application of vitamin did not hasten flowering and it did not influence colour and quality as well as number and size of the flowers in cosmos.

Conversely, Hitchcock and Zimmerman (1941) observed beneficial effect of thiamine on aster plants. Weekly treatment with the vitamin produced taller plants with more fresh weight than control receiving only tap water.

Minnum (1941a) reported the effect of crystalline vitamin B_1, B_2 and B_6 and Vita Flor on the growth of cauliflower and radish in sand culture. Vita Flor contained 0.1% vitamin B_1, 0.5% nicotinic acid and traces of vitamin B_2, B_6 and pantothenic

acid. These substances were given with Hoagland's nutrient solution plus trace elements. None of the treatments was found to have significant effect on the performance of these vegetables. Subsequently, in an extended field trial, Minnum (1941b) again found that crystalline vitamins, Vita Flor and brewer's yeast (containing vitamin B_1, B_2, B_6 etc.) did not exert beneficial effect on the yield of different vegetables, including beets, cauliflower, musk melon, pepper, radish, rutabages, snap beans, summer squash, sweet corn and tomato.

Murneek (1941) conducted two parallel experiments on tomato, dill, Rudbeckia, cosmos and ornamental peppers in pots under greenhouse conditions. The plants were grown on poor and rich soil, as expressed by the presence of organic matter. Of the plants included in the study, tomato was regarded as non-responsive to vitamin B_1, and cosmos as highly responsive. In one experiment, all plants received vitamin B_1 at concentrations of 0.025 and 1.25 mg/gallon (0.005mg/l and 0.278mg/l respectively) of water. In a parallel experiment, the same plants were supplied with leaf mould that contained appreciable amounts of the vitamin. The composition of soil of each pot in both experiments was in the ratio of 1/3 loam: 1/3 sand:1/3 leaf mould. Supply of vitamin B_1 exerted a conspicuous beneficial (though variable) effect on growth of these plants, particularly influencing mostly the roots, so that probably the entire plant body was consequently benefitted.

Spreading of 1/2 to 3/4 inch leaf mould on the soil surface proved good; and sometimes yielded better results than those with vitamin B_1, as leaf mould might have also provided other organic stimulants. Therefore, he concluded that in horticultural practices, success with vitamin B_1 and other growth factors would seem to depend largely upon the kind of soil and type of fertilisers used.

On the other hand, Templeman and Pollard (1941) did not observe any beneficial effect of thiamine and nicotinic acid on the growth of oats and tomato in sand culture. They were of the opinion that the plants were self-sufficient in synthesising these substances for their normal growth and development. Similarly, Clark (1942) found that application of vitamin B_1, with nutrient solution, had no beneficial effect on the growth of Agrostis tenuis and Brassica alba in pure quartz, sand or soil under the experimental conditions.

Minarik (1942) reported similar observations on rice plants. Initially, he gave 0.01ppm synthetic thiamine hydrochloride with nutrient solution for two months and subsequently the amount was increased to 0.1mg/d till completion of the experiment. However, there was no difference between treated and untreated plants as far as colour, lodging or tillering were concerned. He assigned rice to the group containing plants that synthesise sufficient vitamin B_1 to meet their needs.

Gisier (1944) studied the effect of vitamin B_1 on numerous crops, including sunflower, maize, wheat, and flax in pot culture. These plants did not respond to the application of the vitamin.

Mariat (1944) applied vitamin B_1 at the rate of 2.6mg/l with symbiotic, jellied and sugared Knop's solution and compared the performance of treated, cattleya orchid seedlings wih those receiving no vitamin. It was observed that 42% of the total seedlings receiving vitamin B_1 produced leaves in 3 months, while only 3% of untreated seedlings could do so.

De Capite (1949) observed that thiamine, riboflavin and nicotinamide, supplemented with nutrient solution, enhanced the respiratory activity in one-leaf seedlings of wheat, oats and barley. He presumed that these vitamins promoted the catabolic process of the plant cells.

Iijima (1952) investigated physiological changes in Kentucky Wonder and Masterpiece kidney bean, Golden Bantam maize and Miyashige Japanese radish associated with vitamin B_1 treatment. The seeds of these crops were soaked for 24h in different concentrations of vitamin B_1 ranging from 0.0000001 to 100ppm. Among these concentrations, 0.01ppm proved optimum. In general, vitamin application enhanced the germination and accelerated the growth of plumules and radicles, especially at an early germination stage. Moreover, the application of this vitamin increased the

per cent germination of old seeds. It was also observed that, when seeds were grown in the dark, the vitamin content and dry matter of cotyledon, plumule and radicle were decreased as germination progressed. However, the vitamin content was increased on exposing the seeds to light when it got transformed into ester form. Conversely, the concentration of the vitamin and its ester form was lowered in 1 to 4 year old seeds. The application of 0.05ppm vitamin B_1 also increased respiration as well as vitamin B_1 concentration in cotyledons by 150-180% over the control.

Brusca and Haas (1957) studied the effect of several chemically pure salts of organic compounds on citrus in sand culture. Addition of vitamin B_6 (0.01 and 0.02 g/plant) and vitamin B_{12} (0.02 g/plant) to the nutrient solution stimulated the growth of citrus plants.

Barbieri (1959) observed that application of vitamin B_1 and B_6 enhanced plant height, leaf number, fresh and dry weight of pea, broad bean, beet and wheat in pot culture. The effect of both vitamins was most pronounced on beet and poorest on pea. For example, each of these vitamins at 0.01mg/l increased the leaf number by 25% in beet seedlings.

Vergnano (1959) tried vitamins B_1 and B_6 for improving rooting in cuttings of some plants in sand culture. Each vitamin was added at the rate of 0.01mg/l with the nutrient solution.

Vitamin treatments did not improve rooting in Colutea arborescens but Hedera helix and Rosa showed good response. Treated plants of Rosa also produced greater number of buds and leaves with broader leaf blades than the controls.

Školnik and Davydova (1962) applied 100mg each of vitamin B_1 and B_6/l to the roots of tomato plants. They observed that the application of these vitamins averted zinc deficiency symptoms in the plants to a large extent. The plants appeared similar to those which received zinc in their nutrition.

Kjelvick (1965) studied the effect of soaking vegetable seeds in nicotinic acid on yield performance of these crops. He observed that yield of radish and outdoor cucumber was enhanced as a result of soaking their seeds for 24h in nicotinic acid.

Davydova (1966) observed the inter-relationship between zinc and vitamins in the metabolism of tomato plants in soilless culture experiments. Zinc deficiency resulted in a slight reduction of pyridoxine content in roots of tomato plants, but the thiamine and riboflavin contents remained unaltered. In addition, its deficiency also caused the accumulation of nitrate which was lowered on supplying the plants with thiamine and pyridoxine. However, these vitamins did not affect the activity of nitrate reductase.

Aĭzikovick (1967) found that treating rice seeds with vitamin B_1 and B_2 resulted in high rate of germination in field conditions, which consequently enhanced the seed yield by 2% over the control plants.

Gašparíková (1967) investigated the effect of thiamine and β-indole acetic acid as well as of their interaction on the growth and respiration of pea plants. Thiamine and β-indole acetic acid were supplied at the rate of $4x10^{-3}M$ and $10^{-5}M$ solutions to 10-d old seedlings respectively. Both treatments inhibited root as well as shoot growth. However, the effect of thiamine was more pronounced than that of β-indole acetic acid. These compounds together acted synergistically. Respiration of root was also inhibited during the first 8h by individual as well as combined application of these compounds. However, it was later stimulated by β-indole acetic acid and inhibited by thiamine as well as by combined application of both substances.

Gūtmanis (1967) noted that soaking of pea seeds in 0.1% vitamin C and 0.01% vitamin B_1 solution enhanced the seed yield by 14 and 15% respectively. Similarly, vitamin B_1, vitamin C and nicotinic acid increased vitamin C and sugar content of the crop by 12 and 40% respectively.

Mel'Tser (1967) obtained variable respone of two spring wheat cultivars (Krasnozernaya and Remo) in field trials to 0.01% nicotinic acid solution in which the seeds were soaked. The growth

of Krasnozernaya was increased at an early stage, while in the case of Remo, the vitamin was effective at later stage. In general, the number of florets/spikelet was increased in both the cultivars. Moreover, the difference between treated and untreated plants was noteworthy, though, it was small.

Ovcharov and Kulieva (1968) soaked cotton seeds in 0.01% pyridoxine solution for 1-3h after which the seeds were sown with different forms of nitrogen and phosphorus fertilisers. In general, the vitamin slightly promoted germination and increased the area of first leaf two-to three-fold. The effectiveness of vitamin B_6 depended upon the form of fertilisers used. Root length of treated seedlings in the presence of ammonium sulphate was 49% more than in untreated controls and was 14% less than in the presence of calcium nitrate. Pyridoxine increased the nitrogen and phosphorus contents in 2d old seedlings more in the presence of potassium dihydrogen orthophosphate than in that of superphosphate. The soaking of seeds in the same concentration of nicotinic acid was effective irrespective of the type of the fertilisers.

Dimitrova-Russeva and Lilova (1969) studied the effect of the application of thiamine, pyridoxine and nicotinic acid on the uptake of nitrogen and phosphorus by Mentha piperita in nutrient solution as well as in soil. They found that uptake was increased by the application of these vitamins, phosphorus

responding particularly to single application of nicotinic acid and double application of others. Nicotinic acid also enhanced the yield of essential oil as did two applications of thiamine, whereas pyridoxine reduced it.

Zavenyagina and Bukin (1969) investigated the effect of the application of pyridoxine on the germination rate and growth of root and shoot of wheat and pea seedlings. Reduced germination rate and growth of root and shoot, showing the symptoms of vitamin B_6 avitaminosis, were eliminated to a great extent by the application of pyridoxine to the plants with nutrient medium. Chlorophyll content of the leaves was also increased by the application of vitamin B_6 group (pyridoxal and pyridoxamine).

Genkeĭ (1970) investigated the effect of soaking wheat seeds in 0.005, 0.05 and 0.5% nicotinic acid solution and 0.05 and 0.5% nicotinamide solution. The seeds were soaked for 12h and sown in the field. Grain yield was enhanced from 16.7 q/ha in the control to 21.9, 18.8 and 24.5 by the application of 0.005, 0.05% nicotinic acid and of 0.5% nicotinamide respectively, while grain yield was reduced to 14.3 q/ha due to application of 0.5% nicotinic acid. Further, the seeds produced by the plants which were raised from the seeds treated with various concentrations of the vitamin possessed 89.2, 100.2, 96.3, 87.9 and 80.3mg protein/g dry matter respectively, compared with 82.7mg in the control. It indicated that all the treatments of nicotinic acid increased the

seed protein but the application of nicotinamide had no effect or decreased the seed protein in comparison with control.

Mullick and Chatterji (1971) found that niacin at a concentration of 250ppm had no effect on seed germination or hypocotyl growth but inhibited the growth of seedling roots. Moreover, lower concentration of niacin also proved ineffective for germination and growth.

Serebryakova (1971) treated seeds of Rosa cinnamomea with solutions of nicotinic acid (0.01%) or vitamin B_1 (0.02%). Seeds treated with the vitamins showed 3-4d earlier germination than controls. Moreover, the emergence of seedlings was 43 and 53% more in 0.01% (nicotinic acid) and 0.02% (vitamin B_1) solution respectively in comparison with control. Further, treatment with vitamins B_1, B_2, C or nicotinic acid promoted growth as well as synthesis of vitamin C and benefitted quality of plant and production of hips.

Gopala Rao (1973) noted a beneficial effect of riboflavin on 5-6d old seedlings of Phaseolus radiatus in a water culture experiment. Riboflavin significantly enhanced growth, respiration, chlorophyll and protein content of the seedling. For example, riboflavin treated seedlings contained 18.92 and 13.90% more chlorophyll on fifth and sixth day than those of controls. He presumed that riboflavin, being a member of the B-vitamins, accelerated growth, chlorophyll and protein synthesis as a coenzyme of flavin nucleotides.

Gopala Rao et al. (1974) observed high succinic dehydrogenase activity in root and shoot, and enhanced respiration and protein synthesis in 4d old seedlings of Phaseolus radiatus in a water culture experiment as a result of supplying the plants with biotin, pyridoxine, niacin and thiamine in the nutrient solution.

Mikhailova (1974) noted in a field trial that soaking of seeds in nicotinic acid enhanced the growth of and tolerance against flooding in barley. Moreover, treatment with the vitamin also averted the ill effects of flooding on grain yield.

Polyanskaya and Kuvadov (1974) observed the beneficial effect of nicotinic acid on the performance of cotton crop. They reported an increase in growth, nitrogen and phosphorus uptake, number of bolls per plant, boll weight and cotton yield as a result of soaking the cotton seeds in nicotinic acid solution before sowing.

Sinkovics (1974) applied seven different B-vitamins to two cultivars of Capsicum through seed soaking. These vitamins promoted seed germination, seedling growth and earliness, size and quality of the crop. Treatment with nicotinic acid resulted in early maturation of the crop accompanied by high yield.

Radzevičius and Bluzmanas (1975) studied the effect of thiamine and nicotinic acid on tomato in water culture and under

field conditions. These vitamins stimulated root growth followed by an increase in adsorbing surface area by 48 to 52%. In field trial, plants grown from the vitamin soaked-seeds gave greater leaf chlorophyll and vitamin C content, and produced 23.1 to 30.2% more yield than the control plants.

Kulieva et al. (1976) investigated the response of melon and water melon to vitamin treatments in laboratory and field conditions. Seeds of these plants were treated with various concentrations ranging from 0.01 to 0.0001% of thiamine, cyanocobalamine, nicotinic acid, pyridoxine or ascorbic acid. The effect of these compounds on stem and root development as well as on number and weight of fruit was studied in 45 and 90d old plants respectively. Generally, the best results were obtained by treating the seeds with thiamine (0.001%), cyanocobalamine (0.0001%) or nicotinic acid (0.0001%).

Reda et al. (1977) observed the physiological changes in Ammi visnaga associated with seed treatment with thiamine and ascorbic acid. Thiamine and ascorbic acid were given at the concentration of 50 and 50 or 100mg/l. Thiamine promoted root, stem, leaf, umbel and fruit growth and increased the levels of total chromones and their main components, khallin and visnagin, in the fruits. Similarly, ascorbic acid stimulated growth and increased fruit chromone content.

Serebryakova and Kalanova (1977) noted that soaking of rose seeds in nicotinic acid, vitamin B_1, B_2 and C enhanced germination by 43, 51, 30 and 30% respectively over the control. These treatments resulted in vigorous plants and consequently improved their quality. Vitamin B_1 enhanced ascorbic acid formation in young plants by 50% and B_2 and nicotinic acid, by 25%. Similarly, treatments of cuttings with nicotinic acid and vitamin B_1 resulted in a 7-10 fold increase in the number of cuttings exhibiting rooting.

Afridi *et al.* (1979) screened a number of common vitamins and phytohormones with respect to seed germination and *in vitro* radicle growth of barley and noted vitamin B_6 to be the most effective. On the basis of this preliminary trial, they performed an experiment on barley var. K-672/28 in sand culture. Seeds of barley were soaked for 24h in 0.1, 0.3 and 0.5% aqueous pyridoxine solution and thereafter sown in pots. Treatment with pyridoxine benefitted most of the root, shoot and ear characteristics as well as grain yield and quality. Generally, soaking in 0.3 and 0.5% proved equally effective and optimum. Root length and number of lateral roots as well as leaf number were found to be 6.6, 19.0 and 13.5% more in 0.3% treatment than in control. Tiller number/plant was statistically equal in 0.3 and 0.5% treatments, being enhanced by 13% over the control. The same treatment with 0.3% pyridoxine increased shoot length by 5.8% over the control, with 0.5% following closely behind. Both

these treatments consequently resulted in 15% more dry weight than in the control. Similarly, other characteristics, including seed yield and seed carbohydrates were enhanced by 9.0 and 1.4% respectively by 0.3% treatment over the control. But straw yield was 12.7% more in 0.5% than the control, which proved at par with 0.3%.

Pandev (1979), performing a sand culture experiment, observed the effect of indolyl 3-acetic acid, vitamin B_1 and nitrogen and of their interaction on the pattern of nitrogen uptake in wheat. In general, nitrogen uptake progressively increased upto ear formation and decreased later at flowering when plants were supplied with 1-, 2-, 4- or 8-strength of nitrogen in complete Hoagland and Arnon solution. Application of vitamin B_1 and indolyl 3-acetic acid increased the uptake of nitrogen at flowering stage. Thus, dry matter and grain yields were optimum with 4-strength nitrogen which was subsequently improved on inclusion of vitamin B_1 and indolyl 3-acetic acid.

Ahmad et al. (1981) studied the effect of soaking the seeds in pyridoxine solutions (0.02, 0.10 and 0.50%) on tiller number, leaf number at heading and milky grain stages in five cultivars of barley, namely NP-13, NP-21, K-571/10, K-572/28 and Clipper in a factorial randomised field trial. Treatment with 0.1% proved optimum for these characteristics. In case of various cultivars of barley, Clipper and K-572/28 produced maximum and

minimum number of tillers and leaves respectively at all stages but the latter had the tallest plants, while NP-21 gave maximum fresh and dry weight, followed by K-572/28. Treatment x variety effect differed from character to character but the interaction 0.02% x K-572/28 in geneeral proved the best.

Further, Ahmad *et al.* (1982) reported their observations on grain yield and straw yield of the same barley varieties as a result of seed treatment with pyridoxine. Grain and straw yield were recorded to be maximum in 0.1 and 0.02% treatments respectively. Similarly, K-572/28 and NP-21 produced highest grain and straw respectively. As far as interactions were concerned, the combinations 0.1% x K-572/28 and 0.02% x NP-13 proved optimum for these two parameters respectively. The net profit/ha due to 0.1% pyridoxine treatment was calculated to be Rs.572.20.

Ashfaq *et al.* (1983) in a field trial studied the effect of soaking the grains for 12h in 0.0, 0.1, 0.2, 0.3 and 0.4% aqueous pyridoxine solution on seed germination and yield characteristics of triticale var. Bronco-90. The vitamin treatment significantly affected most yield attributes. Among different treatments soaking in 0.2% proved optimum. This treatment enhanced grain yield by 37.7% presumably due to its beneficial effect on grain weight and per cent germination.

Khan and Ansari (1984), working with Phaseolus radiatus, found that soaking of seeds for 10h in pyridoxine solutions (0.0, 0.1, 0.2 and 0.3%) stimulated growth of 10d old seedling (two-leaf stage) in sand culture. Soaking in 0.1% enhanced the fresh weight and number of lateral roots by 18.23 and 23.80% respectively over the water-soaked control.

Gopala Rao and Raghava Reddy (1985) observed the effect of B-vitamins on the uptake of sodium, potassium, calcium and phosphorus in one week old Vigna radiata seedlings. Treatment with the vitamins promoted the uptake of these elements variably. Thiamine and biotin were found ineffective in the uptake of phosphorus; but riboflavin, pyridoxine and pantothenic acid increased the uptake of sodium, potassium and calcium in addition to phosphorus in the seedlings. Application of pyridoxine, pantothenic acid and nicotinic acid particularly showed more influence on potassium and phosphorus uptake than the other vitamins included in the study.

2.4.3 Vitamins applied to tops of plants

Regarding the spraying of B-vitamins on standing crops, less attention has been paid so far. However, in most of the cases, foliar application of these vitamins improved the performance of the crops and enhanced the yield of economically valuable ingredients considerably. The publications on this aspect are meagre and the available work has been reviewed below.

Iijima (1955) observed a beneficial effect of spraying thiamine solution on the leaves of sweet potatoes. Foliar application of thiamine stimulated root growth and increased the number and length of roots accompanied with high percentage of large storage roots. The fresh weight of stem and leaves was also increased by the treatment. However, spraying was more effective in the first half than in the later half of the growing season. The most suitable concentration of thiamine solution was found to be 1ppm, when 2ml of this solution was sprayed/plant at intervals of 10d.

Čajlahjan (1956) studied the effect of vitamin B_1, vitamin C and nicotinic acid on plant growth and development. The vitamins were applied to plants by vacuum-infiltration or spray. Of these, vitamin B_1 accelerated flowering in Perilla and maize.

Iijima (1956a) found that spray of 1 or 100ppm solution of thiamine promoted root growth and leaf respiration in potato, sweet potato and kidney beans. As their respiratory quotient was unity, it appeared that thiamine accelerated the catabolism of leaf-carbohydrate. Iijima (1956b) noted an increase in sugar, starch and thiamine contents of bean seeds as a result of thiamine spray. Similarly, carbohydrate content and C/N ratio of stems, leaves and roots was enhanced by the vitamin, suggesting that the treatment stimulated root development and flower bud differentiation and curtailed the growing period.

In addition, Iijima (1957a) reported that photosynthesis was increased in kidney beans and cabbages by foliar application of vitamin B_1. Iijima (1957b) studied the influence of the spray of thiamine mixed with various agricultural chemicals on garden crops. Combination of thiamine with acidic chemicals or with urea resulted in high yield responses of the crops but in the presence of alkaline chemicals it decreased the yield.

Boukin (1958) reported growth stimulating effect of nicotinic acid or thiamine spray on beans, tobacco, tomato and mulberry. He applied 0.001% solution of these vitamins. The same concentration of the vitamins was also found to promote leaf growth and to enhance root yield by more than 50% in carrot.

Langridge and Brock (1961), working with albino tomato plants, found that such plants were incapable of synthesising thiamine. They applied 2mg of thiamine/100ml water thrice a week to the leaves of these mutants. It was observed that these albino plants changed and resembled their wild type. The treated plants showed normal chlorophyll formation, flowering and seed production, but their progeny remained mutant in phenotype. It was also noted that when albino plants were provided δ-aminolevulenic acid and prophobilinogen, the plants did not assimilate these compounds into chlorophyll, suggesting that the deficiency of thiamine presumably impaired the synthesis of chlorophyll before the formation of pyrrole rings. Further, supply of thiamine precursors

(thiazole and pyrimidine) individually or in combination showed that mutant plants responded only to pyrimidine, indicating a hindrance in the synthesis of this compound.

Kŭdrev and Pavlov (1965) averted ill effect of flooding at tillering, shooting and heading stages in wheat through foliar spray of vitamin B_6 solution. The spray of the vitamin solution also corrected disturbed nitrogen metabolism, particularly when very little damage had been done, and consequently enhanced the grain yield.

Artimonov (1966) found that foliar application of 20mg gibberellin/l inhibited the synthesis of chlorophyll and increased its decomposition in leaves of sugar beet. However, spraying with vitamin B_2 (50mg/l) enhanced the chlorophyll content and corrected the ill effect of gibberellin.

Galachalova *et al.* (1967) separated two types of wheat seeds cultivated in the Novosibrisk province. Seeds obtained from the northern part of province contained less thiamine and more riboflavin and were of lower sowing quality than those used in the southern part. Pre-sowing treatment of the former seeds with thiamine promoted germination and enhanced seed yield of better quality. The same results were obtained when the crops raised from these seeds were sprayed with thiamine.

Kŭdrev and Pandev (1967) noted an increase in nitrogen content in wheat plants from seedling stage to ear emergence. The increase was slow at first and rapid at five leaf stage. A second slight increase in accumulation was observed during flowering and grain development. Spraying with thiamine or pyridoxine accelerated temporary uptake of nitrogen without altering the general trend of uptake.

Popova et al. (1971) sprayed the pistils of Capsicum annuum just after pollination with thiamine, riboflavin, pyridoxine and nicotinic acid in various combinations. These vitamins increased fruit set and number of seeds/fruit, shortened the growing period and increased plant height in F_1-generation. All the vitamins, except riboflavin, caused earliness in the F_1.

Arsen'eva (1977) observed the effect of various physiologically active substances, including B-vitamins (thiamine, nicotinic acid and pyridoxine) and vitamin C on initiation and completion of flowering, flower size and colour and shoot growth in lilac, hydrangea and spiraea. The treatments were given as spray during reproductive organ differentiation and flower bud development. The plants responded differently to these substances in various concentrations. Among these plants, lilac gave the best response for all parameters studied to 100mg pyridoxine spray, while a mixture of thiamine, nicotinic acid and vitamin C at the concentration of 100mg/l proved optimum for spiraea.

El-Kholy and Saleh (1980) sprayed Matricaria chamomilla with 10ml solution each of thiamine and vitamin C (25, 50, 100 and 150ppm strength) at 30 and 45d after transplantation. Of these, 150ppm of either thiamine or vitamin C solution significantly increased number of flowers and head production/plant by 47.22 and 56.60% respectively in first growing season over the control; but essential oil and chamazulene percentage in treated plants remained unaltered. However, the total average yield of these economically important ingredients was increased by the application of 150ppm of either of these vitamins.

To sum up entire review of literature, some inferences can be drawn. Early studies regarding B-vitamins in relation to plants were aimed to investigate suitable media for culturing excised organs and were thus confined to the laboratory. Soon it was realised that the vitamins were indispensable for normal growth and development of excised roots. These researches continued vigorously till the middle of the present century. Consequently, it prompted some workers to try these substances on intact plants under greenhouse and field conditions and they sometimes got encouraging results, but comparatively little attention was paid to this aspect. Among B-vitamins, pyridoxine remained neglected in comparison with other members of this group. Moreover, leguminous crops particularly lentil (Lens culinaris L. Medic.) and summer moong (Vigna radiata L. Wilczek) have not been studied in relation to pyridoxine in the field.

CHAPTER - 3

MATERIALS AND METHODS

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MATERIALS AND METHODS

The field experiments on lentil (Lens culinaris L.Medic.) var. T-36 reported and discussed in this thesis, were conducted in the "rabi" (winter) season of 1982-83, 1983-84 at the University Farm and those on summer moong (Vigna radiata L.Wilczek) var. K-851, in the "zaid" (summer) season of 1983, 1984 at the Botanical Garden of the Aligarh Muslim University, Aligarh (U.P.).

3.1 Agro-climatic conditions

Aligarh is one of the fifty seven districts of Uttar Pradesh (North India). It has an area of $5.024 \times 10^9 m^2$ and is situated at 27° 52' N latitude, 78° 51' E longitude and 187.45m altitude. Its climate is characteristic of western Uttar Pradesh, i.e. semi-arid and sub-tropical climate with hot dry summers and cold winters. The winter extends from the middle of October to the end of March. The mean temperature for December and January, the coldest months, is about $15^\circ C$ and $13^\circ C$ and the extreme minimum record for any single day is $2^\circ C$ and $0.5^\circ C$ respectively. The summer is hot, the average temperature for May is $34.5^\circ C$ and for June $34^\circ C$ whereas the extreme maximum record is $45^\circ C$ and $45.5^\circ C$ respectively. The average annual rainfall is 847.3mm. More than 85% of the total rainfall occurs during June-September and some 10% in the winter which benefits "rabi" crops. These data were

recorded at the Meteorological Observatory, Department of Physics, Aligarh Muslim University, Aligarh.

Aligarh district possesses various types of soil, like sandy, loamy, sandy loam and clay loam.

3.2 Soil characteristics

Before sowing, soil samples were collected from each plot upto a depth of about 10-15cm. These were mixed thoroughly to get a composite sample. The composite sample was analysed in the Soil Chemistry Laboratory of the Indian Agricultural Research Institute (I.A.R.I.), New Delhi. The physico-chemical properties of the soil for each experiment have been given in Table 1.

3.3 Field preparation

Prior to each trial, the experimental field was thoroughly ploughed to ensure maximum soil aeration. It also helped in eliminating the weeds. Plots of $5m^2$ size for each treatment were prepared and irrigated lightly before sowing, to maintain proper moisture regime in the sub-surface of soil. The crop (lentil or moong) was cultivated by using standard agricultural practices. A uniform basal fertiliser dose of nitrogen, phosphorus and potassium was broadcast in each plot to maintain the fertility of the soil. However, basal dose of fertiliser was varied for these crops, depending upon their requirement.

Table 1. Physico-chemical characteristics of surface soil of the fields used for Experiments 1-6.

Characteristics	Year					
	1982-83	1982-83	1983-84	1983	1983	1984
	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5	Experiment 6
1. Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
2. Particle size distribution						
Sand %	72.80	72.80	73.00	76.35	77.81	77.00
Silt %	8.32	7.84	8.67	7.69	7.69	8.50
Clay %	18.88	19.36	18.33	15.96	14.40	14.50
3. pH (1:2)	7.9	8.1	8.1	8.2	8.2	8.2
4. Conductivity E.C. (1:2) (m mhos/cm)	0.45	0.38	0.38	0.35	0.35	0.38
5. Available nitrogen (kg N/ha)	220.34	215.35	240.61	203.76	205.29	216.37
6. Available phosphorus (kg P/ha)	18.90	20.61	18.90	30.34	30.86	28.56
7. Available potassium (kg K/ha)	689.08	695.00	665.12	744.04	750.69	724.39

3.4 Seeds

Authentic seeds of lentil and moong were obtained from the National Seed Corporation, I.A.R.I., New Delhi and their viability was tested by standard methods. The seeds, after surface-sterilisation and soaking treatment, were inoculated with rhizobium prior to sowing by using the method with some modifications as described by Subba Rao (1972). The rhizobium culture for lentil and moong was obtained from the local Government Seed Store, Aligarh. The inoculum was prepared by dissolving 400g colourless gum arabic (coating material) and 100g sugar in 1l of warm water. The solution was cooled and a packet of rhizobium (containing 200g bacterial culture in peat) was added to it and mixed well. A muddy solution resulted. It was used to inoculate 10kg seeds of either lentil or moong depending upon the nature of the bacterial culture mixed in the inoculum medium. Seeds were vigorously mixed with the inoculum until they were evenly covered and moistened by it. These inoculated seeds were spread on clean blotting paper in shade to let the coating get hard. Thereafter, they were sown in the field.

3.4.1 Lentil

Three experiments were conducted on lentil (*Lens culinaris* L.Medic.) var. T-36 during "rabi" seasons of 1982-83 and 1983-84. Of these, the first two experiments were

conducted simultaneously in 1982-83 and the last in the following season.

3.4.1.1 Experiment 1

The first experiment was conducted at the University Farm during 1982-83. The physico-chemical properties of the experimental plot have been given in Table 1.

The aim of this trial was to investigate the effect of soaking the seeds of lentil in graded concentrations of aqueous pyridoxine solution on growth and yield performance of the crop. The range of concentrations of pyridoxine solution and optimum period for soaking had been determined earlier in the laboratory in a preliminary investigation.

Seeds of uniform size were sorted out. After surface-sterilisation with ethyl alcohol, 75g seeds were soaked for 12h in various concentrations of aqueous pyridoxine hydrochloride solution (Table 2) and kept in separate conical flasks.

After soaking, the seeds were inoculated with rhizobium as described previously. Soaked seeds were sown behind the plough in eight rows in $5m^2$ plot at the rate of 50kg/ha on November 5, 1982. The rows were 25cm apart and seeds/row were kept uniform. Each treatment was replicated thrice. The experiment was laid out according to simple randomised block design. A recommended

Table 2. Scheme of treatments for Experiment 1 (Lentil).

S.No.	Treatments	Remarks
1	S_0	Unsoaked seeds
2	S_W	Seeds soaked in water
3	S_1	" " " 0.1% pyridoxine solution
4	S_2	" " " 0.2% " "
5	S_3	" " " 0.3% " "
6	S_4	" " " 0.4% " "
7	S_5	" " " 0.5% " "

N.B. Seeds were soaked for 12h and then treated with rhizobium inoculum

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

basal dose of combined chemical fertilisers (45kg N, 30kg P and 30kg K/ha) was supplied to the field before sowing in the form of commercial grade urea, monocalcium superphosphate and muriate of potash respectively. The experimental field was irrigated twice between sowing and harvesting. Weeding was done once during the entire course of crop development.

Plants were sampled at 60, 90 and 120d after sowing for assessing the growth performance of the crop. At harvest (140d) various yield parameters, seed yield and seed quality were studied.

3.4.1.2 Experiment 2

This experiment was conducted simultaneously with Experiment 1. The physico-chemical properties of the soil are given in Table 1.

The object of the experiment was to study the effect of foliar application of aqueous pyridoxine hydrochloride solution and to determine the best stage for the foliar application with regard to growth and yield performance of lentil. The foliar application of pyridoxine hydrochloride solution was done either at flower- or fruit-initiation. The thirteen spray treatments selected for the study were applied in a simple randomised block design (Table 3).

Table 3. Scheme of treatments for Experiment 2 (Lentil).

S.No.	Treatments	Remarks
1	F_0	Untreated plants
2	$F_{W(90)}$	Foliar spray of water at 90d
3	$F_{1(90)}$	" " " 0.1% pyridoxine at 90d
4	$F_{2(90)}$	" " " 0.2% " " "
5	$F_{3(90)}$	" " " 0.3% " " "
6	$F_{4(90)}$	" " " 0.4% " " "
7	$F_{5(90)}$	" " " 0.5% " " "
8	$F_{W(110)}$	" " " water at 110d
9	$F_{1(110)}$	" " " 0.1% pyridoxine at 110d
10	$F_{2(110)}$	" " " 0.2% " " "
11	$F_{3(110)}$	" " " 0.3% " " "
12	$F_{4(110)}$	" " " 0.4% " " "
13	$F_{5(110)}$	" " " 0.5% " " "

N.B. Seeds were treated with rhizobium inoculum

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

Unsoaked seeds inoculated with rhizobium were sown behind the plough in eight rows of 5m^2 plot at the rate of 50kg/ha on November 10, 1982. There were three replicates for each treatment. The same basal dose of chemical fertilisers as in Experiment 1 was supplied uniformly. Other agricultural practices were kept same as in the other experiment. Each plot was sampled at 120d after sowing for growth performance. Yield attributes and seed quality were studied at harvest (140d).

3.4.1.3 Experiment 3

This field trial was conducted the next year, i.e. in the "rabi" season of 1983-84 in the same field that was used in the previous two experiments. The soil characteristics of the field are given in Table 1.

The trial was based on the findings of Experiment 1 and 2. The object of the experiment was to observe the combined effect of soaking and spray of pyridoxine hydrochloride solution and to determine the optimum combination for growth and yield performance of lentil. The foliar application in this experiment was done at flower-initiation.

The experiment was conducted according to simple randomised block design by taking sixteen combinations of treatments as given in Table 4. Each treatment was replicated thrice. The seeds were soaked for 12h in aqueous pyridoxine

Table 4. Scheme of treatments for Experiment 3 (lentil).

S.No.	Treatments	Remarks	
		Seeds soaked in	Foliar spray of
1	$S_W + F_W$	Water	Water
2	$S_W + F_1$	"	0.1% pyridoxine
3	$S_W + F_2$	"	0.2% "
4	$S_W + F_3$	"	0.3% "
5	$S_2 + F_W$	0.2% pyridoxine	Water
6	$S_2 + F_1$	" "	0.1% pyridoxine
7	$S_2 + F_2$	" "	0.2% "
8	$S_2 + F_3$	" "	0.3% "
9	$S_3 + F_W$	0.3% "	Water
10	$S_3 + F_1$	" "	0.1% pyridoxine
11	$S_3 + F_2$	" "	0.2% "
12	$S_3 + F_3$	" "	0.3% "
13	$S_4 + F_W$	0.4% "	Water
14	$S_4 + F_1$	" "	0.1% pyridoxine
15	$S_4 + F_2$	" "	0.2% "
16	$S_4 + F_3$	" "	0.3% "

N.B. Seeds were soaked for 12h and then treated with rhizobium inoculum

Plants were sprayed at 90d

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

hydrochloride solution and later inoculated with rhizobium prior to sowing. Other agricultural practices including basal fertiliser dose, irrigation, weeding etc. were also similar to the previous two experiments.

Plants from each plot were collected at 60, 90 and 120d after sowing for assessing growth performance. At harvest (140d) yield parameters and seed quality were studied.

3.4.2 Summer Moong

As in the case of lentil, two field experiments were conducted simultaneously on moong (Vigna radiata L.Wilczek) var. K-851 during "zaid" season of 1983 and a third field experiment, in 1984.

3.4.2.1 Experiment 4

This experiment was laid out after preliminary studies in the laboratory in which the period for soaking the seeds in various concentrations of aqueous pyridoxine hydrochloride solution was standardised.

The aim of the trial was to observe the effect of soaking the seeds in different concentrations of aqueous pyridoxine hydrochloride solution on growth and yield performance of moong.

The experiment was conducted at the Botanical Garden of the University according to a simple randomised block design. Seeds of uniform size were selected. After surface sterilisation with ethyl alcohol, 30g seeds were soaked for 4h in various concentrations of aqueous pyridoxine hydrochloride solution (Table 5) and kept in separate conical flasks.

After soaking, the seeds were inoculated with rhizobium as described in Section 3.4 and sown behind the plough in 6 rows in $5m^2$ plot at the rate of 20kg/ha on April 1, 1983. The rows were 33cm apart and the number of seeds was kept approximately uniform in each row. There were three replicates for each treatment. A recommended uniform basal dose of 10kg N, 30kg P and 35kg K/ha was supplied to the field, before sowing, in the form of commercial grade urea, monocalcium superphosphate and muriate of potash respectively. The field was irrigated thrice between sowing and harvesting. Weeding was done twice during the entire period of crop growth. The physico-chemical properties of the soil are given in Table 1.

The plants were sampled at 20, 30, 40 and 50d after sowing for growth analysis, while at harvest (62d), various yield parameters and seed quality were studied.

Table 5. Scheme of treatments for Experiment 4 (Summer moong)

S.No.	Treatments	Remarks
1	S_W	Seeds soaked in water
2	S_1	" " " 0.1% pyridoxine solution
3	S_2	" " " 0.2% " "
4	S_3	" " " 0.3% " "
5	S_4	" " " 0.4% " "
6	S_5	" " " 0.5% " "

N.B. Seeds were soaked for 4h and then treated with rhizobium inoculum

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

3.4.2.2 Experiment 5

This trial was conducted simultaneously with Experiment 4. The physico-chemical properties of the soil are given in Table 1.

The object of the trial was to investigate the effect of foliar application of aqueous pyridoxine hydrochloride solution on growth and yield performance of moong. The various concentrations of pyridoxine hydrochloride solution were applied to the leaves either at flower- or fruit-initiation. The experiment was also laid out according to simple randomised block design and the scheme of treatments is given in Table 6.

Unsoaked seeds inoculated with rhizobium,as described in Experiment 4, were sown in the field on April 5, 1983. All the agricultural practices including basal fertiliser dose, irrigation, weeding etc. were same as in Experiment 4. Each treatment was replicated thrice.

Sampling was done at 45 and 55d after sowing for growth analysis, and at harvest (62d), for yield parameters and seed quality.

3.4.2.3 Experiment 6

This trial was conducted in the "zaid" season 1984. The physico-chemical properties of the soil are given in Table 1.

Table 6. Scheme of treatments for Experiment 5 (Summer moong).

S.No.	Treatments	Remarks
1	$F_{W(35)}$	Foliar spray of water at 35d
2	$F_{1(35)}$	" " " 0.025% pyridoxine at 35d
3	$F_{2(35)}$	" " " 0.050% " " "
4	$F_{3(35)}$	" " " 0.10% " " "
5	$F_{4(35)}$	" " " 0.20% " " "
6	$F_{W(45)}$	" " " Water at 45d
7	$F_{1(45)}$	" " " 0.025% pyridoxine at 45d
8	$F_{2(45)}$	" " " 0.050% " " "
9	$F_{3(45)}$	" " " 0.10% " " "
10	$F_{4(45)}$	" " " 0.20% " " "

N.B. Seeds were treated with rhizobium inoculum

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

The object of the trial was to investigate the ideal combination of pyridoxine soaking and spray at an appropriate growth stage for obtaining optimum performance of moong. The combinations of soaking and foliar treatments were made on the basis of the observation made in Experiments 4 and 5. The details of the treatments selected in this investigation are shown in Table 7. The experiment was conducted according to a simple randomised block design.

After soaking the seeds in pyridoxine solution, they were inoculated with rhizobium and sown behind the plough in 6 rows in $5m^2$ plots at the rate of 20kg/ha on April 5, 1984. Other agricultural practices, including basal dressing of fertiliser, irrigation, weeding etc., were similar to previous trials on moong (Experiments 4 and 5). Each treatment was replicated three times.

Sampling, to observe growth performance, was done at 45 and 55d after sowing, while yield characteristics and quality of seeds were studied at harvest (62d).

3.5 Sampling technique

Random sample of three plants from each plot at various stages were collected. To count the number of nodules, plants were dug out carefully and washed to wipe away all adhered foreign particles like dust etc. Samplings were used for assessing the

Table 7. Scheme of treatments for Experiment 6 (Summer moong).

S.No.	Treatments	Remarks	
		Seeds soaked in	Foliar spray of
1	$S_W + F_{W(35)}$	Water	Water
2	$S + F_{W(35)}$	0.3% pyridoxine	"
3	$S + F_{1(35)}$	" "	0.1% pyridoxine
4	$S + F_{2(35)}$	" "	0.2% "
5	$S + F_{3(35)}$	" "	0.3% "
6	$S_W + F_{W(45)}$	Water	Water
7	$S + F_{W(45)}$	0.3% pyridoxine	"
8	$S + F_{1(45)}$	" "	0.1% pyridoxine
9	$S + F_{2(45)}$	" "	0.2% "
10	$S + F_{3(45)}$	" "	0.3% "

N.B. Seeds were soaked for 4h and then treated with rhizobium inoculum

Plants were sprayed either at 35 or 45d

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

following parameters for growth performance. At maturity, all the plants from each plot were harvested and thrashed to get seed yield.

3.6 Growth parameters

To assess the effect of pyridoxine on growth, the following parameters were studied:

(a) Plant length
(b) Root length/plant
(c) Root nodule number/plant
(d) Leaf number/plant
(e) Fresh weight/plant
(f) Dry weight/plant

3.7 Net assimilation rate (NAR)

NAR was calculated according to the following formula as described by Milthorpe and Moorby (1979):

$$NAR = \frac{(W_2-W_1)}{(t_2-t_1)} \times \frac{(\ln L_2-\ln L_1)}{(L_2-L_1)}$$

$$\text{or; } NAR = \frac{(W_2-W_1)}{(t_2-t_1)} \times \frac{2.303\ (\log_{10} L_2-\log_{10} L_1)}{(L_2-L_1)}$$

Here; W_1 = dry weight of whole plant at I growth stage

L_1 = leaf area of whole plant at I growth stage

t_1 = days of sampling at I growth stage

W_2 = dry weight of whole plant at II growth stage

L_2 = leaf area of whole plant at II growth stage

t_2 = days of sampling at II growth stage

ln = logarithm to base e

$\log_{10}$ = logarithm to base 10

3.8 Yield parameters

The following parameters were studied for yield assessment at the time of harvest:

(a) Pod number/plant

(b) Pod length

(c) Seed number/pod

(d) 1,000 seed weight

(e) Seed yield

3.9 Chemical analyses

(i) Total pyridoxine content in seeds of both the crops was estimated before sowing them in the field.

(ii) Nitrate reductase activity (NRA) in leaves was measured at various stages of growth on fresh weight basis.

(iii) Nitrogen, phosphorus and potassium in leaves were estimated at various stages of growth on dry weight basis.

(iv) For assessing the quality of seeds, total protein content was estimated at harvest.

3.9.1 Estimation of pyridoxine

Seeds were dried and powdered. The powder was sieved and pyridoxine content was estimated colorimetrically according to the method of Hochberg et al. (1944a,b) which is described below.

3.9.1.1 Preparation of seed extract

1g seed powder was taken in a 20ml calibrated test tube. 10ml of 4N-hydrochloric acid was added. The test tube was placed in a water bath and heated for 1h. The contents of the tube were stirred occasionally which helped in hydrolysing the bound pyridoxine as well as in extracting the vitamin. The solution was cooled and the pH was adjusted to 3 with 1N-sodium hydroxide and hydrochloric acid. At this pH, 3ml of buffer (sodium citrate) was mixed followed by the addition of 2.5g of Fuller's earth (formerly called Llyod's reagent). The tube was stoppered and shaken occasionally for 5min. The suspension was centrifuged and supernatant was discarded. The residue was washed with 15ml of

acidulated water. 5ml of 2N-sodium hydroxide solution was added to the residue and the final volume was made upto 20ml with distilled water. The suspension was dispersed for a period of 3min by frequent inversions of the tube and centrifuged. 10ml of the eluate was mixed with 50ml of isopropanol and was again centrifuged. The clear supernatant was decanted and pH was adjusted to 5-7 by using a few drops of 12N-hydrochloric acid. This extract was used for pyridoxine estimation.

3.9.1.2 Colour development

The following tubes were setup in order to estimate the pyridoxine contents in the seeds:

Test tube 1 : 6ml test-extract + 2ml of ammonia-ammonium chloride solution + 1ml of boric acid solution.

Test tube 2 : 6ml of test-extract + 2ml of ammonia-ammonium chloride solution + 1ml of distilled water.

Test tube 3 : 6ml of test-extract + 2ml of ammonia-ammonium chloride solution + 1ml of standard pyridoxine hydrochloride solution (10 µg).

In each test tube, 1ml of 2,6 dichloroquinone chloroimide solution was added. Test tube 1 acted as the blank. The optical density was read at 660nm on "Spectronic-20"

colorimeter exactly after 1min of addition of 2,6 dichloroquinone chloroimide reagent. The pyridoxine content in seeds was calculated by using the following formula:

$$\frac{L_2}{L_3-L_2} \times \frac{10}{6} \times \frac{60}{10} \times \frac{18.5}{W} = \mu g \text{ pyridoxine/g seed powder}$$

In the above equation:

L_2 = represents optical density of the solution present in Test tube 2.

L_3-L_2 = represents increase in optical density due to the 10 µg pyridoxine added in Test tube 3.

W = stands for weight of seed powder used.

$\frac{60 \times 18.5}{10}$ = is used for dilution factor

3.9.2 Estimation of NRA

NRA was estimated at all growth stages in fresh leaves. Random samples of leaves from each plot were taken and cut into small pieces. The enzyme activity was determined according to the method of Jaworski (1971), briefly described below:

500mg leaf pieces were weighed and placed in polythene vials. 2.5ml of phosphate buffer and 0.5ml of 0.2M-potassium nitrate solution were added followed by addition of 2.5ml of

5% isopropanol. Lastly, two drops of chloramphenicol solution were poured to avoid bacterial growth in the medium. These vials were incubated for 2h in dark at 30°C.

3.9.2.1 Colour development

0.4ml of incubation mixture was taken in a test tube to which 0.3ml of 1% sulphanilamide and 0.02% N-1-nephthyl ethylene diamine hydrochloride (NED HCl) were added. The solution was left for 20min for maximum colour development.

It was diluted to 5ml with sufficient amount of distilled water and optical density was read at 540nm using a "Spectronic-20" colorimeter. A blank, consisting of 4.4ml of distilled water plus 0.3ml each of sulphanilamide and NED HCl, was used simultaneously.

A standard curve was plotted by taking various concentrations of potassium nitrite. The optical density of the samples was compared with this calibrated curve and NRA was expressed as n mol NO_2^-/h/g fresh leaf tissue.

3.9.3 Estimation of NPK

Three plants from each plot were randomly chosen and dried in an oven for 24h. Healthy leaves were plucked, powdered and passed through a 72 mesh screen. Nitrogen, phosphorus and potassium were estimated as described below:

3.9.3.1 Digestion of leaf powder

Leaf powder was digested for leaf nitrogen, phosphorus and potassium according to Lindner (1944). 100mg of dry leaf powder was taken in a 50ml Kjeldhal flask. 2ml of chemically pure sulphuric acid was added and heated for 2h. Heating with acid turned the contents black. After cooling for 15min, 0.5ml of chemically pure 30% hydrogen peroxide was added drop by drop. The solution was again heated for about 30min till the colour became light yellow. It was then cooled and 3-4 drops of hydrogen peroxide were added. The contents were again heated for about 15min to get a clear solution. Excess of hydrogen peroxide was avoided as it may oxidise ammonia in the absence of organic matter. The peroxide-digested material was transferred into a 100ml volumetric flask with three or four washings with distilled water and volume was made upto the mark.

3.9.3.1.1 Estimation of nitrogen

Lindner's method (1944) was adopted for the estimation of nitrogen in the samples.

A 10ml aliquot of the peroxide digested material was taken in 50ml volumetric flask. 2ml of 2.5N sodium hydroxide and 1ml of 10% sodium silicate solutions were added to neutralise excess of acid and to prevent turbidity respectively. The volume

of the solution was made upto the mark with the help of distilled water. In a 10ml graduated test tube, 5ml aliquot of this solution was taken and 0.5ml of Nessler's reagent was added and mixed. The final volume was made up with distilled water. After waiting for 5min to get optimum colour development, optical density of the solution was determined at 525nm on a "Spectronic-20" colorimeter. A blank consisting of distilled water and Nessler's reagent was run simultaneously. A standard curve of known dilutions of ammonium sulphate solution was plotted. The reading of each sample was compared with this calibration curve and nitrogen in leaves was estimated in terms of percentage on dry weight basis.

3.9.3.1.2 Estimation of phosphorus

Total phosphorus in the sulphuric acid-peroxide digest was estimated by the method of Fiske and Subba Row (1925). A 5ml aliquot was taken in a 10ml graduated test tube and 1ml of molybdic acid (2.5% ammonium molybdate in 10N-sulphuric acid) was added carefully followed by the addition of 0.4ml of 1-amino-2-naphthol-4-sulphonic acid. The colour turned blue. Distilled water was used to make up the volume to 10ml. The solution was shaken, kept for 5min and then transferred to a colorimetric tube. The optical density was read at 620nm on a "Spectronic-20" colorimeter. A blank was used simultaneously with each determination. The standard curve was prepared by using known

concentrations of monobasic potassium phosphate solution. The readings of samples were compared with this curve and phosphorus content in leaves was computed in terms of percentage on dry weight basis.

3.9.3.1.3 Estimation of potassium

Potassium content was estimated flame photometrically. A 10ml aliquot was taken and it was read by using the filter for potassium. A blank was run side by side. The readings were compared with a calibration curve plotted from known dilutions of a standard potassium sulphate solution. The potassium in leaves was expressed as per cent on a dry weight basis.

3.9.4 Estimation of protein

The protein content of seeds was extracted according to Yih and Clark (1965) and estimated by the method of Lowry et al. (1951). Sufficient amount of seed powder was spread over a sheet of paper and dried overnight in an oven at 80°C. The dried samples were cooled in a dessicator for about 5min before weighing. 50mg of each sample was taken and transferred to a mortar. 1ml of cold 5% trichloroacetic acid was added to it. The powder was ground well and homogenate was collected in a centrifuge tube, with repeated washings with trichloroacetic acid. The volume was made upto 5ml with 5% trichloroacetic acid. It was kept for 1h to

allow the complete precipitation of proteins. The samples were then centrifuged at 4,000rpm for 15min and supernatant was discarded. To the residue, 5ml of 1N-sodium hydroxide solution was added and shaken well for complete mixing. It was kept for half an hour on a water bath at 60°C so as dissolve the precipitated proteins completely. After cooling for 15min, the mixture was centrifuged at 4,000rpm for 15min and the supernatant, containing the protein, was collected. It was then diluted with appropriate quantity of water and used for estimation of total protein in the seed.

3.9.4.1 Colour development

1ml of diluted aliquot was taken in a test tube. 5ml of reagent B was added and left for 10min. Afterward, 0.5ml of Folin's reagent was added with immediate mixing and kept for half an hour for optimum colour development. The optical density of each sample was measured at 660nm on a "Spectronic-20" colorimeter. A blank containing distilled water, reagent B and Folin's reagent was used simultaneously with each sample. The reading was compared with a calibration curve, obtained by using known dilutions of standard egg albumen solution.

The reagents used in analysing various constituent of plant parts in section 3.9 were prepared as described in the appendix.

3.10 Statistical analysis

All the data were analysed statistically according to Panse and Sukhatme (1967). The "F" test was applied to assess the significance of the data at 5% level of probability. The error due to replicates was also determined. The models of the analysis of variance(ANOVA) are given in Table 8. Critical difference (C.D.) was calculated to compare the effect of various treatments, using the following formula:

$$C.D. = \sqrt{\frac{\text{Standard Error} \times 2}{\text{Replicates}}} \times t\ 5\%\ \text{(from table)}$$

Association of various growth parameters, NAR, NRA and NPK content in leaves with seed yield and seed protein was determined by computing correlation coefficients (r). The significance of correlation coefficient values at 5% level of probability was determined. For these calculations, the following formulae were used:

$$r = \frac{\Sigma(x-\bar{x})(y-\bar{y})}{\sqrt{(x-\bar{x})^2 \times (y-\bar{y})^2}}$$

x = independent character ($\bar{x}$ = mean)

y = dependent character ($\bar{y}$ = mean)

Table 8. Models of analysis of variance (ANOVA).

Experiment 1 (Simple randomised block design)

Source of variation	D.F.	S.S.	M.S.S.	F
Replications	2			
Treatments	6			
Error	12			
Total	20			

Experiment 2 (Simple randomised block design)

Source of variation	D.F.	S.S.	M.S.S.	F
Replications	2			
Treatments	12			
Error	24			
Total	38			

Contd. Table 8. Models of analysis of variance (ANOVA).

Experiment 3 (Simple randomised block design)

(a) at 60 and 90d

Source of variation	D.F.	S.S.	M.S.S.	F
Replications	2			
Treatments	3			
Error	6			
Total	11			

(b) at 120d and harvest

Source of variation	D.F.	S.S.	M.S.S.	F
Replications	2			
Treatments	15			
Error	30			
Total	47			

Experiment 4 (Simple randomised block design)

Source of variation	D.F.	S.S.	M.S.S.	F
Replications	2			
Treatments	5			
Error	10			
Total	17			

Contd. Table 8. Models of analysis of variance (ANOVA).

Experiment 5 (Simple randomised block design)

(a) at 45d

Source of variation	D.F.	S.S.	M.S.S.	F
Replications	2			
Treatments	5			
Error	10			
Total	17			

(b) at 55d and harvest

Source of variations	D.F.	S.S.	M.S.S.	F
Replications	2			
Treatments	9			
Error	18			
Total	29			

Contd. Table 8. Models of analysis of variance (ANOVA).

Experiment 6 (Simple randomised block design)

(a) 45d

Source of variations	D.F.	S.S.	M.S.S.	F
Replications	2			
Treatments	6			
Error	12			
Total	20			

(b) at 55d and harvest

Source of variation	D.F.	S.S.	M.S.S.	F
Replications	2			
Treatments	9			
Error	18			
Total	29			

$$\text{Test of significance } (t') = r \times \sqrt{\frac{n-2}{1-r^2}}$$

n = number of total observations, i.e. number of treatments x replicates.

When t' value was found greater than the predicted t value obtained from the table at 5% level of probability, the correlation coefficient (r) value was declared to be significant.

CHAPTER - 4

EXPERIMENTAL RESULTS

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EXPERIMENTAL RESULTS

4.1 Experiment 1

In this simple randomised field trial, the effect of pre-sowing seed treatment with graded aqueous pyridoxine solution, viz. S_0 (unsoaked), S_W (water soaked), S_1 (0.1%), S_2 (0.2%), S_3 (0.3%), S_4 (0.4%) and S_5 (0.5%), was studied on growth characteristics, net assimilation rate, leaf nitrate reductase activity, leaf NPK content, seed yield and seed protein content of lentil var. T-36. Since S_0 and S_W were at par for most of the characteristics, the values obtained in optimum treatment were compared with water-soaked control (S_W). The data are described below and summarised in Tables 9-13.

4.1.1 Growth characteristics

Six growth parameters, namely plant length, root length, root nodule number, leaf number, fresh weight and dry weight were studied at 60, 90 and 120d after sowing. All these parameters, except root length at 90 and 120d, were significantly affected by pyridoxine application (Tables 9-10).

4.1.1.1 Plant length

Soaking in 0.1% pyridoxine solution (S_1) produced tallest plants at all three stages (Table 9). The value given by

Table 9. Effect of pre-sowing seed treatment with pyridoxine on plant length, root length and root nodule number of lentil var. T-36 (Mean of three replicates).

Treatments	Plant length (cm)			Root length/plant (cm)			Root nodule number/plant		
	Days after sowing								
	60	90	120	60	90	120	60	90	120
S_0	24.955	43.867	56.889	12.166	12.545	9.500	12.970	15.008	3.022
S_W	24.633	41.556	56.668	11.733	11.112	8.085	13.140	14.904	3.281
S_1	28.644	50.587	59.000	12.733	10.276	7.683	14.142	15.409	3.392
S_2	26.878	44.878	58.889	12.634	13.156	8.556	16.692	18.210	3.895
S_3	26.878	46.000	56.933	11.656	12.278	7.655	18.260	21.462	4.339
S_4	26.578	44.000	57.443	12.600	13.467	9.221	11.589	12.777	4.091
S_5	25.144	43.556	47.311	9.777	12.389	7.201	10.914	11.206	3.112
C.D. at 5%	0.889	2.536	1.288	0.307	N.S.	N.S.	0.352	0.339	0.468

N.B. Seeds were soaked for 12h and then treated with rhizobium inoculum

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

N.S. Non-significant

this treatment differed critically from all other treatments at all stages, except at 120d where it was equal to that for S_2. The increase in S_1 was 16.28% at 60d, 21.73% at 90d and 4.11% at 120d compared with the plant length in S_W. Treatment S_5 produced shortest plants and the value differed significantly from those for all other treatments.

4.1.1.2 Root length/plant

Root length was significantly affected only at 60d (Table 9). S_1 gave maximum value for this parameter but its effect was equal to those of S_3 and S_4. Treatment S_1 enhanced root length by 8.52% over S_W. Soaking in the highest concentration of pyridoxine (S_5) proved inhibitory for this character.

4.1.1.3 Root nodule number/plant

Optimum root nodules were recorded in S_3 at 60 and 90d (Table 9). The increase due to this treatment over S_W was 38.96% at 60d and 44.00% at 90d respectively. At 120d also, optimum root nodules were found in S_3 but its effect was at par with those of S_2 and S_4. Treatment S_3 increased the root nodules by 32.25% over S_W. At 60 and 90d, S_5 produced significantly lowest number of root nodules; but at 120d, its effect was at par with those of S_0 and S_W.

4.1.1.4 Leaf number/plant

At 60d, significant maximum leaf number was given by S_1. This treatment increased leaf number by 8.61% over S_W. At 90 and 120d, treatment S_3 resulted in highest number of leaves and its value differed significantly from those for all other treatments. The increase due to S_3 was 35.84% at 90d and 52.33% at 120d in comparison with the leaf number in water soaked control (S_W). S_5 at 60 and 90d produced lowest leaf number; but at 120d, it gave more leaves than S_0 and S_W (Table 10).

4.1.1.5 Fresh weight/plant

At 60d, treatment S_1, being at par with S_2 and S_3, gave the highest value for fresh weight and differed critically from the remaining treatments (Table 10). It increased the fresh weight by 14.74% over the water soaked-control (S_W). The highest concentration of pyridoxine, i.e. 0.5% (S_5), gave the lowest value and thus proved inhibitory for fresh weight. At 90 and 120d, treatment S_3 gave significantly highest fresh weight and increased it by 15.00% at 90d and 54.66% at 120d over the water-soaked control (S_W). At both these stages also, treatment S_5 gave the lowest values for fresh weight.

Table 10. Effect of pre-sowing seed treatment with pyridoxine on leaf number, fresh weight and dry weight of lentil var. T-36 (Mean of three replicates).

Treatments	Leaf number/plant			Fresh weight/plant (g)			Dry weight/plant (g)		
	Days after sowing								
	60	90	120	60	90	120	60	90	120
S_0	24.778	59.778	115.444	1.633	8.111	15.778	0.373	1.407	5.194
S_W	25.167	57.667	121.333	1.791	8.889	17.889	0.375	1.526	5.302
S_1	27.333	68.750	141.556	2.055	9.000	22.333	0.438	1.802	7.015
S_2	25.333	65.667	170.333	1.962	9.000	22.667	0.449	1.806	7.010
S_3	25.333	78.333	184.833	1.822	10.222	27.667	0.411	2.073	8.131
S_4	25.222	61.778	128.111	1.778	8.500	14.889	0.399	1.710	5.091
S_5	23.556	54.778	131.889	1.371	6.889	14.467	0.352	1.377	4.755
C.D. at 5%	0.762	2.858	8.618	0.233	0.811	2.268	0.024	0.165	0.311

N.B. Seeds were soaked for 12h and then treated with rhizobium inoculum

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

4.1.1.6 Dry weight/plant

At 60d, maximum dry weight was recorded in treatment S_2. The value given by this treatment significantly differed from those for all other treatments, except S_1. The increase due to S_2 was 19.73% over water soaked control (S_W). Treatment S_5 showed poorest effect. At 90 and 120d, S_3 gave significantly maximum value for this parameter. The increase due to this treatment was 35.84% at 90d and 53.36% at 120d over S_W. At these two stage also, S_5 gave lowest value (Table 10).

4.1.2 Net assimilation rate (NAR)

NAR was determined for 60-90 and 90-120d periods and was noted to be significantly affected by pyridoxine treatment of seeds (Table 11). Treatment S_3 exhibited maximum NAR at both intervals. It increased NAR by 18.45% at 60-90d and 15.59% at 90-120d over S_W. The value given by this treatment was significantly different from all other treatments at the first interval (60-90d); but, at the second interval (90-120d), it was equal to the values given by S_1 and S_2. In addition, NAR due to S_5 fell below that in the controls at both intervals but was at par with that in S_4 at the 90-120d interval.

Table 11. Effect of pre-sowing seed treatment with pyridoxine on net assimilation rate (NAR) and nitrate reductase activity (NRA) of lentil var. T-36 (Mean of three replicates).

Treatments	NAR ($x10^{-4}$ g/cm^2/d)		NRA (n mol NO_2^-/g/h)		
	Days interval		Days after sowing		
	60–90	90–120	60	90	120
S_0	4.590	3.819	107.139	74.136	76.528
S_W	4.841	4.073	107.616	71.745	75.093
S_1	4.596	4.422	121.968	77.485	90.877
S_2	5.367	4.418	133.924	86.094	102.834
S_3	5.734	4.708	147.852	102.834	110.009
S_4	4.564	3.634	137.750	81.311	90.877
S_5	3.946	3.369	125.552	81.311	88.485
C.D. at 5%	0.266	0.315	2.948	2.946	2.561

N.B. Seeds wore soaked for 12h and then treated with rhizobium inoculum

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

4.1.3 Nitrate reductase activity (NRA)

Leaf NRA was measured at 60, 90 and 120d after sowing (Table 11). Pre-sowing seed treatment with various concentrations of pyridoxine solution significantly enhanced leaf NRA at all stages. Among various treatments, S_3 proved optimum and the value given by this treatment differed critically from those given by all other treatments. This treatment enhanced NRA by 37.39% at 60d, 43.33% at 90d and 46.50% at 120d in comparison with NRA in S_W.

4.1.4 Leaf NPK content

Leaf NPK content was estimated at 60, 90 and 120d after sowing. The data at all stages generally showed significant effect of pyridoxine treatment (Table 12) and are described below in detail.

4.1.4.1 Nitrogen

Soaking in 0.3% solution (S_3) gave significantly maximum values for leaf nitrogen content at all stages, except at 60d where the value was equal to that for S_4. The increase due to S_3 was 1.65% at 60d, 1.35% at 90d and 0.80% at 120d in comparison with the nitrogen content in S_W. On the other hand, S_5 gave significantly lowest values for leaf nitrogen content at all stages (Table 12).

Table 12. Effect of pre-sowing seed treatment with pyridoxine on leaf NPK content of lentil var. T-36 (Mean of three replicates).

Treatments	Nitrogen (%)			Phosphorus (%)			Potassium (%)		
	Days after sowing								
	60	90	120	60	90	120	60	90	120
S_0	2.800	2.350	1.950	0.520	0.432	0.320	5.346	3.560	2.485
S_W	2.950	2.600	2.150	0.480	0.416	0.312	5.438	3.651	2.556
S_1	3.750	2.850	2.300	0.551	0.486	0.360	5.860	4.060	3.043
S_2	4.150	3.400	2.250	0.600	0.518	0.408	6.500	5.180	3.860
S_3	4.600	3.950	2.950	0.632	0.538	0.440	7.160	5.690	4.267
S_4	4.450	3.000	1.550	0.499	0.480	0.336	6.234	4.468	3.750
S_5	2.300	1.750	1.500	0.458	0.452	0.285	4.045	2.550	2.009
C.D. at 5%	0.447	0.447	0.314	0.042	0.025	0.027	0.287	0.288	0.272

N.B. Seeds were soaked for 12h and then treated with rhizobium inoculum

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

4.1.4.2 Phosphorus

Soaking in 0.3% (S_3) gave maximum values for leaf phosphorus content at 60 and 90d (Table 12). However, the value given by this treatment was statistically equal to that for S_2 at each of these stages. Treatment S_3 enhanced leaf phosphorus content by 0.15% at 60d and 0.12% at 90d over S_W. At 120d also, S_3 gave optimum leaf phosphorus value which differed critically from those for the remaining treatments. This treatment increased leaf phosphorus content by 0.13% over S_W. The highest concentration of pyridoxine solution (S_5) gave the lowest values for leaf phosphorus content at all stages of sampling.

4.1.4.3 Potassium

For leaf potassium content, S_3 gave significantly optimum values at all three stages that differed critically from those for the remaining treatments (Table 12). This treatment enhanced leaf potassium content by 1.72% at 60d, 2.04% at 90d and 1.71% at 120d compared with the respective values in S_W. Treatment S_5 giving significantly lowest values for leaf potassium content, proved inhibitory at all three stages.

4.1.5 Yield characteristics

Five yield characters (pod number/plant, pod length, seed number/pod, 1,000 seed weight and seed yield) were studied

at harvest. All these parameters, except pod length, were significantly affected by pyridoxine treatment. The data are described below and condensed in Table 13.

4.1.5.1 Pod number/plant

Pod number was maximum in treatment S_3. The value given by this treatment was at par with that for S_2. An increase of 50.26% was recorded in pod number due to S_3 in comparison with S_W. The highest concentration of pyridoxine solution, i.e. S_5, produced significantly lowest number of pods (Table 13).

4.1.5.2 Pod length

As mentioned earlier, pre-sowing seed treatment with pyridoxine did not show significant effect on pod length (Table 13).

4.1.5.3 Seed number/pod

Seed number was maximum in treatment S_3. The value given by this treatment differed critically from those for the remaining treatments, except S_1. The increase due to S_3 was 14.80% over S_W. S_5 produced minimum number of seeds; but the value was at par with those for S_4 and S_W (Table 13).

Table 13. Effect of pre-sowing seed treatment with pyridoxine on yield parameters and seed protein content of lentil var. T-36 (Mean of three replicates).

Treatments	Pod number/ plant	Pod length (cm)	Seed number/ pod	1,000 seed weight (g)	Seed yield (q/ha)	Protein content (%)
S_0	59.422	1.003	1.700	20.200	13.400	20.985
S_W	61.078	1.117	1.655	20.233	13.420	21.036
S_1	71.711	1.003	1.822	19.400	13.800	22.177
S_2	91.488	1.019	1.806	19.467	15.540	22.899
S_3	91.778	1.012	1.900	19.367	15.800	23.244
S_4	61.756	1.027	1.666	20.100	15.400	21.883
S_5	51.733	1.034	1.600	19.733	13.010	21.343
C.D. at 5%	2.374	N.S.	0.086	0.626	0.444	0.393

N.B. Seeds were soaked for 12h and then treated with rhizobium inoculum

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

N.S. Non-significant

4.1.5.4 1,000 seed weight

1,000 seed weight was adversely affected by pyridoxine treatment (Table 13). Among various treatments, S_3 produced seeds of lowest weight. However, the value given by this treatment did not differ critically from those for S_5, S_2 and S_1. The decrease in seed weight due to S_3 was 4.28% compared with that in S_W.

4.1.5.5 Seed yield

Maximum seed yield was obtained in treatment S_3 (Table 13). The value given by this treatment was critically different from those for the remaining treatments, except S_2 and S_4. Treatment S_3 enhanced seed yield by 17.73% over S_W. The lowest seed yield was recorded in S_5. The value given by this treatment was, however, statistically equal to those for S_0 and S_W.

4.1.6 Seed protein

Seed protein content was significantly enhanced by all pyridoxine treatments. Of these, S_3 resulted in maximum seed protein content and differed critically from all other treatments, except S_2. The increase in seed protein content due to S_3 was 2.21% in comparison with S_W (Table 13).

4.2 Experiment 2

In this simple randomised field experiment on lentil var. T-36, the treatments consisted of foliar spray at 90 or 110d of 0.0%, 0.1%, 0.2%, 0.3%, 0.4% and 0.5% aqueous pyridoxine solution and were designated as $F_{W(90)}$, $F_{1(90)}$, $F_{2(90)}$, $F_{3(90)}$, $F_{4(90)}$, $F_{5(90)}$, $F_{W(110)}$, $F_{1(110)}$, $F_{2(110)}$, $F_{3(110)}$, $F_{4(110)}$ and $F_{5(110)}$ respectively. An unsprayed treatment (F_0) was also included.

The same parameters as in Experiment 1 were studied. All the controls, i.e. F_0, $F_{W(90)}$ and $F_{W(110)}$ proved at par with each other for all parameters. Therefore, the values obtained in the optimum treatment were compared with the respective water-sprayed control only. The data are presented in Tables 14-18 and described below.

4.2.1 Growth characteristics

In this experiment, the sampling was done only at 120d to assess growth performance of the crop as affected by spray treatments. The same growth parameters as in Experiment 1 were studied. The data are presented in Tables 14-15 and described below.

4.2.1.1 Plant length

$F_{2(90)}$ produced the tallest plants. The value given by this treatment was ciritically different from those for the other treatments. $F_{2(90)}$ enhanced plant length by 8.32% in comparison with $F_{W(90)}$. On the other hand, $F_{4(110)}$ produced significantly shortest plants (Table 14).

4.2.1.2 Root length/plant

Among various treatments, $F_{5(90)}$ gave maximum value for root length (Table 14). However, the value recorded due to this treatment was at par with those for $F_{4(90)}$, $F_{2(90)}$ and $F_{3(90)}$. The increase in $F_{5(90)}$ was 53.76% and $F_{2(90)}$, 48.75% over $F_{W(90)}$.

4.2.1.3 Root nodule number/plant

The highest number of root nodules was produced by $F_{2(90)}$ and the value recorded for this treatment differed significantly from those for the remaining treatments (Table 14). Treatment $F_{2(90)}$ increased root nodules by 33.99% in comparison with $F_{W(90)}$. Treatment $F_{5(110)}$ resulted in lowest number of root nodules; but the value given by this treatment was at par with that for $F_{4(110)}$.

Table 14. Effect of pyridoxine spray on plant length, root length and root nodule number of lentil var. T-36 (Mean of three replicates).

Treatments	Plant length (cm)	Root length/ plant (cm)	Root nodule number/plant
F_0	60.639	5.584	3.276
$F_{W(90)}$	60.056	5.167	3.266
$F_{1(90)}$	63.667	6.167	4.185
$F_{2(90)}$	65.055	7.686	4.376
$F_{3(90)}$	63.389	7.667	3.656
$F_{4(90)}$	59.889	7.722	2.975
$F_{5(90)}$	58.778	7.945	2.626
$F_{W(110)}$	60.501	5.490	3.362
$F_{1(110)}$	63.389	6.020	3.940
$F_{2(110)}$	63.861	7.083	4.140
$F_{3(110)}$	62.000	6.333	3.064
$F_{4(110)}$	58.055	5.888	2.521
$F_{5(110)}$	55.111	5.844	2.486
C.D. at 5%	0.609	0.438	0.132

N.B. Seeds were treated with rhizobium inoculum

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied.

4.2.1.4 Leaf number/plant

The maximum number of leaves was found in $F_{2(90)}$ and the value recorded for this treatment was critically different from those for the remaining treatments (Table 15). The increase due to this treatment was 48.59% in comparison with $F_{W(90)}$. On the other hand, plants sprayed with $F_{3(90)}$ possessed minimum number of leaves and the value was at par with those for $F_{4(90)}$ and $F_{5(90)}$.

4.2.1.5 Fresh weight/plant

Among various treatments, $F_{2(90)}$ produced optimum fresh weight of plants (Table 15). The value obtained in this treatment differed critically from those in the remaining treatments. The increase due to $F_{2(90)}$ was 50.22% over $F_{W(90)}$. On the other hand, $F_{5(90)}$ gave significantly lowest value for this parameter.

4.2.1.6 Dry weight/plant

The highest dry weight was recorded in $F_{2(90)}$ and the value was significantly different from those for all other treatments (Table 15). $F_{2(90)}$ enhanced dry weight by 59.87% compared with $F_{W(90)}$. The lowest value for dry weight was given by $F_{5(90)}$ which was equal to that for $F_{4(90)}$.

Table 15. Effect of pyridoxine spray on leaf number, fresh weight and dry weight of lentil var. T-36 (Mean of three replicates).

Treatments	Leaf number/ plant	Fresh weight/ plant (g)	Dry weight/ plant (g)
F_0	104.330	16.500	4.616
$F_{W(90)}$	105.734	16.200	4.593
$F_{1(90)}$	133.222	16.556	5.238
$F_{2(90)}$	157.111	24.333	7.343
$F_{3(90)}$	69.222	12.889	4.298
$F_{4(90)}$	69.333	11.667	3.853
$F_{5(90)}$	72.667	10.167	3.589
$F_{W(110)}$	105.167	15.444	4.731
$F_{1(110)}$	109.667	16.401	4.411
$F_{2(110)}$	113.800	17.444	5.238
$F_{3(110)}$	112.889	13.556	4.634
$F_{4(110)}$	105.333	13.444	4.410
$F_{5(110)}$	103.222	12.889	4.062
C.D. at 5%	5.003	0.774	0.282

N.B. Seeds were treated with rhizobium inoculum

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

4.2.2 Net assimilation rate (NAR)

NAR was estimated only for 90-120d period and was significantly optimum in $F_{2(90)}$. This treatment enhanced NAR by 42.20% over $F_{W(90)}$. Treatment $F_{5(90)}$ gave significantly lowest value (Table 16).

4.2.3 Nitrate reductase activity (NRA)

NRA in leaves was estimated at 120d (Table 16). Among different treatments, $F_{2(90)}$ resulted in maximum enzyme activity. However, the value obtained in this treatment was at par with those in $F_{3(90)}$ and $F_{1(110)}$. The increase due to $F_{2(90)}$ was 16.46% in comparison with $F_{W(90)}$. The lowest enzyme activity was recorded in $S_{5(110)}$; but it was at par with that in $F_{4(110)}$.

4.2.4 Leaf NPK content

Like growth characteristics and NRA, leaf NPK was also estimated at 120d. The NPK content in leaves was found to be significantly affected by pyridoxine spray (Table 17) and the data are described below.

4.2.4.1 Nitrogen

$F_{2(90)}$ resulted in maximum nitrogen content in leaves (Table 17). The value given by this treatment differed

Table 16. Effect of pyridoxine spray on net assimilation rate (NAR) and nitrate reductase activity (NRA) of lentil var. T-36 (Mean of three replicates).

Treatments	NAR ($x10^{-4} g/cm^2/d$)	NRA (n mol $NO_2^-/g/h$)
F_0	3.868	74.136
$F_{W(90)}$	3.768	74.745
$F_{1(90)}$	4.342	80.833
$F_{2(90)}$	5.358	87.051
$F_{3(90)}$	4.568	86.094
$F_{4(90)}$	3.678	81.312
$F_{5(90)}$	3.201	73.133
$F_{W(110)}$	3.684	75.332
$F_{1(110)}$	3.584	86.094
$F_{2(110)}$	4.755	76.561
$F_{3(110)}$	3.917	62.179
$F_{4(110)}$	3.836	59.787
$F_{5(110)}$	3.536	57.729
C.D. at 5%	0.247	2.711

N.B. Seeds were treated with rhizobium inoculum

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

significantly from those for all other treatments, except $F_{1(110)}$. Treatment $F_{2(90)}$ enhanced leaf nitrogen content by 1.25% in comparison with $F_{W(90)}$. Further, $F_{5(110)}$ gave lowest leaf nitrogen and was at par with $F_{5(90)}$.

4.2.4.2 Phosphorus

Like leaf nitrogen, phosphorus content in leaves (Table 17) was maximum in $F_{2(90)}$ and was at par with that in $F_{1(110)}$. Treatment $F_{2(90)}$ increased phosphorus content by 0.08% in comparison with $F_{W(90)}$. Treatment $F_{5(90)}$ gave lowest leaf phosphorus value; but it was equal to those for $F_{5(110)}$ and $F_{4(110)}$.

4.2.4.3 Potassium

Unlike nitrogen and phosphorus, leaf potassium (Table 17) was significantly maximum in treatment $F_{1(90)}$ which was at par with $F_{1(110)}$. Treatment $F_{1(90)}$ increased leaf potassium by 1.35% and $F_{2(90)}$, by 0.90% in comparison with $F_{W(90)}$. Treatment $F_{4(110)}$ gave the lowest value for this parameter but was equalled by $F_{3(110)}$, $F_{5(110)}$ and $F_{5(90)}$.

4.2.5 Yield characteristics

Five yield parameters (pod number/plant, pod length, seed number/pod, 1,000 seed weight and seed yield) were studied at

Table 17. Effect of pyridoxine spray on leaf NPK content of lentil var. T-36 (Mean of three replicates).

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)
F_0	1.800	0.317	2.557
$F_{W(90)}$	1.950	0.304	2.354
$F_{1(90)}$	2.800	0.336	3.706
$F_{2(90)}$	3.200	0.384	3.250
$F_{3(90)}$	2.100	0.304	2.622
$F_{4(90)}$	1.650	0.224	2.245
$F_{5(90)}$	1.100	0.218	2.083
$F_{W(110)}$	1.950	0.307	2.495
$F_{1(110)}$	3.150	0.354	3.437
$F_{2(110)}$	2.250	0.346	2.613
$F_{3(110)}$	1.500	0.320	2.167
$F_{4(110)}$	1.650	0.282	2.011
$F_{5(110)}$	1.050	0.240	2.149
C.D. at 5%	0.291	0.033	0.204

N.B. Seeds were treated with rhizobium inoculum

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

harvest. All of these, except 1,000 seed weight, were significantly affected by pyridoxine spray. The data are summarised in Table 18 and described below.

4.2.5.1 Pod number/plant

$F_{2(90)}$ produced maximum number of pods (Table 18). The value given by this treatment differed significantly from those for all treatments, except $F_{2(110)}$. The increase due to $F_{2(90)}$ was observed to be 35.58% in comparison with $F_{W(90)}$. Treatment $F_{5(110)}$ gave significantly lowest number of pods.

4.2.5.2 Pod length

Maximum pod length (Table 18) was noted in $F_{2(110)}$. The value given by $F_{2(110)}$ was at par with that for $F_{2(90)}$. The increase due to $F_{2(110)}$ was 3.54% in comparison with $F_{W(110)}$. Treatment $F_{5(110)}$ produced the smallest pods. However, the value due to this treatment was at par with that for $F_{4(110)}$.

4.2.5.3 Seed number/pod

$F_{2(110)}$ gave maximum number of seeds (Table 18). However, the value obtained for this treatment was at par with those for $F_{1(90)}$, $F_{2(90)}$ and $F_{1(110)}$. Treatment $F_{2(110)}$ produced 4.57% and $F_{2(90)}$, 3.86% more seeds than $F_{W(110)}$ and $F_{W(90)}$ respectively. Significantly lowest seeds were produced by the plants sprayed with 0.5% pyridoxine at 90d, i.e. $F_{5(90)}$.

Table 18. Effect of pyridoxine spray on yield parameters and seed protein content of lentil var. T-36 (Mean of three replicates).

Treatments	Pod number/ plant	Pod length (cm)	Seed number/ pod	1,000 seed weight (g)	Seed yield (q/ha)	Protein content (%)
F_O	63.222	1.015	1.810	19.567	13.590	21.299
$F_{W(90)}$	61.462	1.008	1.813	19.650	13.520	21.111
$F_{1(90)}$	68.333	1.028	1.872	18.600	14.820	22.678
$F_{2(90)}$	83.333	1.050	1.883	19.267	15.420	22.518
$F_{3(90)}$	48.155	1.000	1.758	19.033	12.593	22.016
$F_{4(90)}$	46.869	1.005	1.781	19.667	13.123	21.421
$F_{5(90)}$	44.333	0.995	1.550	19.757	12.360	21.133
$F_{W(110)}$	62.000	1.017	1.817	19.033	13.937	21.500
$F_{1(110)}$	64.667	1.023	1.839	19.050	14.720	22.328
$F_{2(110)}$	81.988	1.053	1.900	19.233	15.280	21.877
$F_{3(110)}$	47.222	1.001	1.770	18.667	13.190	21.644
$F_{4(110)}$	47.167	0.982	1.732	19.100	11.720	21.233
$F_{5(110)}$	38.333	0.967	1.675	19.933	11.670	20.596
C.D. at 5%	3.801	0.022	0.067	N.S.	0.456	0.390

N.B. Seeds were treated with rhizobium inoculum
A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied
N.S. Non-significant.

4.2.5.4 1,000 seed weight

As mentioned earlier, pyridoxine spray did not significantly affect 1,000 seed weight (Table 18).

4.2.5.5 Seed yield

Significant maximum seed yield (Table 18) was recorded in $F_{2(90)}$; but it was at par with that given by $F_{2(110)}$. Treatment $F_{2(90)}$ enhanced seed yield by 14.05% in comparison with $F_{W(90)}$. Treatment $F_{5(110)}$ gave minimum seed yield and the value was at par with that for $F_{4(110)}$.

4.2.6 Seed protein

Seed protein content was also significantly affected by the foliar application of pyridoxine (Table 18). It was maximum in $F_{1(90)}$. The value obtained in this treatment was equal to those for $F_{2(90)}$ and $F_{1(110)}$. Treatment $F_{1(90)}$ enhanced seed protein content by 1.57% compared with $F_{W(90)}$. Treatment $F_{5(110)}$ produced significantly lowest seed protein content.

4.3 Experiment 3

In this simple randomised field trial on lentil var. T-36, the treatments consisted of sixteen combinations of soaking the seeds for 12h and spray at 90d. The concentrations of aqueous

pyridoxine solution selected for soaking were 0.0%, 0.2%, 0.3% and 0.4% and for foliar spray, 0.0%, 0.1%, 0.2% and 0.3%. These combinations were designated as S_W+F_W, S_W+F_1, S_W+F_2, S_W+F_3, S_2+F_W, S_2+F_1, S_2+F_2, S_2+F_3, S_3+F_W, S_3+F_1, S_3+F_2, S_3+F_3, S_4+F_W, S_4+F_1, S_4+F_2 and S_4+F_3.

The same parameters as in Experiments 1 and 2 were studied. The data are condensed in Tables 19-24 and described below.

4.3.1 Growth characteristics

In this experiment also, the same growth parameters were studied as were included in Experiments 1 and 2. However, the samples collected at 60 and 90d after sowing consisted only of water-sprayed plants that had received various pre-sowing seed treatment as pyridoxine spray was applied at 90d. All soaking and spray treatments were included in the sampling done on 120d. The data (Tables 19-21) indicated that all parameters, except root length at 90d, were significantly affected by the treatments.

4.3.1.1 Plant length

Among the 60d samples, treatment S_2+F_W resulted in significantly tallest plants (Table 19). This treatment enhanced plant length by 4.97% in comparison with S_W+F_W. At this stage, the value given by S_4+F_W was lowest but was at par with those for S_W+F_W and S_3+F_W. At the 90d stage, S_3+F_W gave optimum

Table 19. Combined effect of pyridoxine soaking and spray on plant length and root length of lentil var. T-36 (Mean of three replicates).

Treatments	Plant length (cm)			Root length/plant (cm)		
	Days after sowing					
	60	90	120	60	90	120
S_W+F_W	25.500	41.278	48.333	10.678	10.053	5.100
S_W+F_1	-	-	48.222	-	-	5.889
S_W+F_2	-	-	49.844	-	-	6.733
S_W+F_3	-	-	52.200	-	-	7.933
S_2+F_W	26.767	42.856	53.178	11.189	9.456	5.334
S_2+F_1	-	-	53.778	-	-	5.389
S_2+F_2	-	-	54.044	-	-	6.833
S_2+F_3	-	-	53.578	-	-	5.834
S_3+F_W	25.122	45.011	52.533	10.255	9.533	6.722
S_3+F_1	-	-	54.322	-	-	6.833
S_3+F_2	-	-	49.711	-	-	5.444
S_3+F_3	-	-	49.444	-	-	5.611
S_4+F_W	24.80	40.800	52.200	10.533	8.889	6.500
S_4+F_1	-	-	49.556	-	-	6.889
S_4+F_2	-	-	49.667	-	-	5.734
S_4+F_3	-	-	48.375	-	-	5.250
C.D. at 5%	0.818	0.937	0.368	0.440	N.S.	0.309

N.B. Seeds were soaked for 12h and then treated with rhizobium inoculum

Plants were sprayed at 90d

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

N.S. Non-significant.

value for this parameter and differed critically from all other treatments. The increase due to this treatment was 9.04% over S_W+F_W. Treatment S_4+F_W, giving lowest plant length, was at par with S_W+F_W. Considering the effect of various treatments at 120d, S_3+F_1 gave maximum plant length. The value recorded for this treatment differed significantly from those for the remaining treatments, except S_2+F_2. Treatment S_3+F_1 increased plant length by 12.39% compared with S_W+F_W. Treatment S_W+F_1 gave shortest plants. The value was, however, at par with those for S_W+F_W and S_4+F_3.

4.3.1.2 Root length/plant

Root length at 60 and 120d only was found significantly affected by various treatments (Table 19). At 60d, S_2+F_W gave significantly optimum value which was 4.78% higher than that for S_W+F_W which gave the lowest value. However, the values obtained in S_W+F_W, S_3+F_W and S_4+F_W were statistically equal at this stage. At 120d, S_W+F_3 gave significantly optimum root length. The value was 55.55% more in comparison with S_W+F_W. Lowest value for this parameter was noted in S_W+F_W and was at par with those for S_2+F_W, S_2+F_1 and S_4+F_3.

4.3.1.3 Root nodule number/plant

At 60 and 90d, S_3+F_W produced significantly highest number of root nodules (Table 20). The increase due to this treatment was 45.49% and 47.45% at 60 and 90d respectively compared with S_W+F_W. At both stages, S_4+F_W gave significantly lowest value for this parameter. However, at 120d, S_2+F_3 exhibited maximum number of root nodules. The value given by this treatment was statistically equal to those for S_2+F_W, S_2+F_1, S_2+F_2 and S_3+F_W. Treatment S_2+F_3 resulted in 49.67% more root nodules than S_W+F_W. Further, S_4+F_3 gave significantly minimum value for this parameter at the last sampling.

4.3.1.4 Leaf number/plant

At 60d, S_2+F_W gave significantly highest number of leaves (Table 20). It enhanced the leaf number by 22.46% in comparison with S_W+F_W. Treatment S_3+F_W resulted in lowest number of leaves which were statistically equal to those for S_W+F_W and S_4+F_W. On the other hand, at 90 and 120d, S_3+F_W manifested significantly highest number of leaves and increased it by 42.76% and 47.10% respectively compared with S_W+F_W. Significantly minimum leaves were recorded in S_W+F_W at 90d. On the other hand at 120d, minimum leaves were produced by S_4+F_3. The value obtained in this treatment differed significantly from those in the rest of the treatments, except S_4+F_2.

Table 20. Combined effect of pyridoxine soaking and spray on root nodule number and leaf number of lentil var. T-36 (Mean of three replicates).

Treatments	Root nodule number/plant			Leaf number/plant		
	Days after sowing					
	60	90	120	60	90	120
S_W+F_W	13.326	13.727	3.467	20.778	33.778	110.400
S_W+F_1	-	-	3.257	-	-	113.018
S_W+F_2	-	-	4.443	-	-	115.400
S_W+F_3	-	-	2.961	-	-	123.999
S_2+F_W	18.233	17.578	4.934	25.444	41.778	105.800
S_2+F_1	-	-	5.005	-	-	127.867
S_2+F_2	-	-	5.004	-	-	148.133
S_2+F_3	-	-	5.189	-	-	109.466
S_3+F_W	19.388	20.241	5.089	18.222	48.222	162.399
S_3+F_1	-	-	3.157	-	-	117.866
S_3+F_2	-	-	2.874	-	-	117.399
S_3+F_3	-	-	2.768	-	-	115.800
S_4+F_W	11.042	12.887	3.431	21.000	41.000	114.533
S_4+F_1	-	-	2.733	-	-	92.600
S_4+F_2	-	-	2.762	-	-	85.599
S_4+F_3	-	-	2.422	-	-	84.320
C.D. at 5%	0.445	0.435	0.307	2.938	1.418	6.354

N.B. Seeds were soaked for 12h and then treated with rhizobium inoculum

Plants were sprayed at 90d

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

4.3.1.5 Fresh weight/plant

At 60d, significantly highest fresh weight was recorded in plants receiving treatment S_2+F_W (Table 21). This treatment resulted in an increase of 27.61% in fresh weight compared with S_W+F_W. On the other hand, S_3+F_W gave lowest value for this parameter. However, the value was equal to that for S_4+F_W. At 90 and 120d, S_3+F_W produced significantly highest fresh weight. It enhanced fresh weight by 63.18% and 60.77% compared with S_W+F_W at the two stages respectively. The lowest value for fresh weight was given by S_W+F_W at 90d and S_4+F_1 at 120d.

4.3.1.6 Dry weight/plant

S_2+F_W produced optimum dry weight at 60d (Table 21). The value differed significantly from those for other treatments. The increase due to S_2+F_W was 34.71% in comparison with S_W+F_W. At this stage, S_3+F_W gave significantly lowest value. However, at 90 and 120d, maximum dry weight was recorded in the plants receiving the treatment S_3+F_W. The values given by other treatments were significantly lower than that for this treatment. The plants treated with S_3+F_W produced 53.17% and 106.90% more dry weight respectively at the two stages than S_W+F_W. Moreover, S_W+F_W gave lowest value for this parameter at both stages, but its effect was at par with those of S_2+F_W and S_4+F_W at 90d and of S_4+F_2 at 120d.

Table 21. Combined effect of pyridoxine soaking and spray on fresh weight and dry weight of lentil var. T-36 (Mean of three replicates).

Treatments	Fresh weight/plant (g)			Dry weight/plant (g)		
	Days after sowing					
	60	90	120	60	90	120
S_W+F_W	1.811	4.889	14.444	0.314	1.200	3.984
S_W+F_1	-	-	15.833	-	-	4.315
S_W+F_2	-	-	19.833	-	-	5.075
S_W+F_3	-	-	20.333	-	-	4.614
S_2+F_W	2.311	5.744	18.000	0.423	1.428	4.591
S_2+F_1	-	-	18.056	-	-	5.164
S_2+F_2	-	-	18.333	-	-	5.161
S_2+F_3	-	-	17.056	-	-	4.890
S_3+F_W	1.322	7.978	23.222	0.266	1.838	8.243
S_3+F_1	-	-	17.500	-	-	5.453
S_3+F_2	-	-	17.222	-	-	5.257
S_3+F_3	-	-	18.556	-	-	5.181
S_4+F_W	1.622	5.667	15.278	0.320	1.307	4.889
S_4+F_1	-	-	10.611	-	-	4.436
S_4+F_2	-	-	11.611	-	-	4.197
S_4+F_3	-	-	12.333	-	-	4.337
C.D. at 5%	0.352	1.299	2.124	0.065	0.334	0.219

N.B. Seeds were soaked for 12h and then treated with rhizobium inoculum

Plants were sprayed at 90d

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

4.3.2 Net assimilation rate (NAR)

NAR was computed for 60-90d and 90-120d periods (Table 22) and S_3+F_W proved significantly optimum for both. It increased NAR by 48.51% at 60-90d and 36.26% at 90-120d compared with S_W+F_W. The lowest value for NAR was noted in S_W+F_W for the first period and S_3+F_3 for the second period. During the first period, the value obtained in S_W+F_W was equal to that in S_4+F_W. The effect of S_3+F_3 on NAR for the second period was at par with those of S_W+F_3 and S_3+F_2.

4.3.3 Nitrate reductase activity (NRA)

Leaf NRA was measured at 60, 90 and 120d. The enzyme activity at all stages was significantly affected by pyridoxine treatments (Table 22). Among various treatments, S_3+F_W manifested significantly highest enzyme activity in leaves at all stages. It enhanced leaf NRA at 60, 90 and 120d by 35.37%, 32.98% and 51.60% respectively in comparison with S_W+F_W. Significantly lowest enzyme activity was recorded in S_W+F_W at 60 and 90d and in S_4+F_3 at 120d.

4.3.4 Leaf NPK content

Leaf NPK was also estimated at 60, 90 and 120d. The pyridoxine treatments significantly affected the content of these nutrients in leaves at all stages. The data are summarised in Table 23 and described below.

Table 22. Combined effect of pyridoxine soaking and spray on net assimilation rate (NAR) and nitrate reductase activity (NRA) of lentil var. T-36 (Mean of three replicates).

Treatments	NAR ($\times 10^{-4}$ g/cm^2/d)		NRA (n mol NO_2^-/g/h)		
	Days interval		Days after sowing		
	60-90	90-120	60	90	120
S_W+F_W	5.219	4.525	103.630	74.934	77.485
S_W+F_1	-	5.032	-	-	86.332
S_W+F_2	-	5.543	-	-	92.876
S_W+F_3	-	4.189	-	-	76.528
S_2+F_W	5.855	4.930	124.356	84.720	99.486
S_2+F_1	-	5.410	-	-	104.029
S_2+F_2	-	4.578	-	-	103.313
S_2+F_3	-	4.937	-	-	97.574
S_3+F_W	7.751	6.166	140.283	99.646	117.470
S_3+F_1	-	4.377	-	-	90.159
S_3+F_2	-	4.028	-	-	80.833
S_3+F_3	-	3.955	-	-	66.962
S_4+F_W	5.546	4.713	127.538	82.905	95.623
S_4+F_1	-	4.674	-	-	86.094
S_4+F_2	-	5.147	-	-	62.178
S_4+F_3	-	4.760	-	-	47.830
C.D. at 5%	0.483	0.236	2.929	2.853	2.631

N.B. Seeds were soaked for 12h and then treated with rhizobium inoculum

Plants were sprayed at 90d

A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

4.3.4.1 Nitrogen

Treatment S_3+F_W gave significantly highest leaf nitrogen content at all samplings, except at 120d where the value was maximum in S_2+F_2 but equalled by S_3+F_W (Table 23). Treatment S_3+F_W increased leaf nitrogen content by 2.10%, 1.00% and 1.16% at 60, 90 and 120d respectively in comparison with S_W+F_W. Significantly lowest nitrogen content was recorded in plants receiving treatment S_W+F_W at 60d, S_4+F_W at 90d and S_4+F_3 at 120d; but the value obtained in S_4+F_3 at the last stage was equal to that for S_4+F_2.

4.3.4.2 Phosphorus

As is evident from Table 23, significantly maximum leaf phosphorus was recorded in S_3+F_W at 60d. It enhanced leaf phosphorus by 0.14%, compared with S_W+F_W, which gave significantly lowest value. At 90d, S_3+F_W gave maximum leaf phosphorus content. However, the value was at par with those for S_2+F_W and S_4+F_W. The increase due to S_3+F_W was 0.10% compared with S_W+F_W. At this stage also, S_W+F_W showed significantly poorest effect on phosphorus content. At 120d, S_2+F_2 manifested maximum leaf phosphorus content (0.12% higher than in S_W+F_W). The value was, however, at par with those for S_W+F_2 and S_3+F_W. Treatment S_4+F_3 gave significantly lowest value for this parameter but was at par with S_4+F_1 and S_4+F_2.

Table 23. Combined effect of pyridoxine soaking and spray on leaf NPK content of lentil var. T-36 (Mean of three replicates).

Treatments	Nitrogen (%)			Phosphorus (%)			Potassium (%)		
	Days after sowing								
	60	90	120	60	90	120	60	90	120
S_W+F_W	2.700	2.500	1.750	0.504	0.454	0.336	5.284	3.257	2.296
S_W+F_1	-	-	1.800	-	-	0.403	-	-	3.498
S_W+F_2	-	-	2.420	-	-	0.440	-	-	3.336
S_W+F_3	-	-	1.500	-	-	0.384	-	-	2.041
S_2+F_W	3.850	3.100	1.620	0.600	6.520	0.384	6.691	4.878	3.336
S_2+F_1	-	-	1.690	-	-	0.418	-	-	3.125
S_2+F_2	-	-	3.220	-	-	0.461	-	-	2.776
S_2+F_3	-	-	1.530	-	-	0.312	-	-	1.990
S_3+F_W	4.800	3.500	2.910	0.648	0.550	0.426	7.273	5.599	3.056
S_3+F_1	-	-	1.530	-	-	0.374	-	-	3.297
S_3+F_2	-	-	1.360	-	-	0.352	-	-	2.150
S_3+F_3	-	-	1.290	-	-	0.278	-	-	2.002
S_4+F_W	3.800	2.150	1.740	0.560	0.518	0.328	4.396	4.425	3.084
S_4+F_1	-	-	1.510	-	-	0.208	-	-	2.450
S_4+F_2	-	-	1.100	-	-	0.208	-	-	2.155
S_4+F_3	-	-	0.770	-	-	0.205	-	-	1.800
C.D. at 5%	0.733	0.342	0.435	0.028	0.036	0.041	0.364	0.334	0.160

N.B. Seeds were soaked for 12h and then treated with rhizobium inoculum
Plants were sprayed at 90d
A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied

4.3.4.3 Potassium

Treatment S_3+F_W manifested significantly highest potassium content at all three stages (Table 23). It enhanced potassium content by 1.99%, 2.34% and 1.66% at 60, 90 and 120d respectively compared with S_W+F_W. The significantly lowest value for this parameter was recorded in S_4+F_W at 60d and in S_W+F_W at 90d. At 120d, S_4+F_3 gave lowest value but it was at par with that for S_2+F_3.

4.3.5 Yield characteristics

The same yield characters were studied at harvest as in the previous two experiments. All the parameters, except 1,000 seed weight, were significantly affected by pyridoxine treatments. The data are summarised in Table 24 and described below.

4.3.5.1 Pod number/plant

Significantly highest number of pods was recorded in treatment S_3+F_W. This treatment enhanced pod number by 74.10% in comparison with S_W+F_W. Among various treatments, S_W+F_W resulted in significantly lowest number of pods (Table 24).

Table 24. Combined effect of pyridoxine soaking and spray on yield parameters and seed protein content of lentil var. T-36 (Mean of three replicates).

Treatments	Pod number/ plant	Pod length (cm)	Seed number/ pod	1,000 seed weight (g)	Seed yield (q/ha)	Protein content (%)
S_W+F_W	65.767	0.992	1.728	20.167	13.460	21.106
S_W+F_1	69.667	1.010	1.773	19.700	14.780	22.600
S_W+F_2	101.778	1.040	1.865	19.600	16.540	22.155
S_W+F_3	100.500	0.996	1.786	20.033	16.820	21.876
S_2+F_W	83.333	1.019	1.829	19.600	16.120	22.237
S_2+F_1	112.556	1.016	1.849	20.000	16.940	22.896
S_2+F_2	107.222	1.011	1.833	19.967	14.540	22.634
S_2+F_3	83.889	1.030	1.793	19.567	17.040	22.097
S_3+F_W	114.500	1.042	1.892	19.600	17.740	23.549
S_3+F_1	92.167	1.028	1.863	19.467	15.340	23.027
S_3+F_2	90.222	1.014	1.818	19.667	15.340	22.460
S_3+F_3	90.167	1.009	1.816	19.533	15.480	22.200
S_4+F_W	79.222	1.002	1.809	19.633	14.180	22.080
S_4+F_1	76.333	0.985	1.781	19.767	15.570	21.566
S_4+F_2	74.500	0.995	1.773	19.733	15.680	21.234
S_4+F_3	71.333	0.983	1.709	19.633	16.120	20.765
Total	0.700	0.019	0.022	N.S.	0.227	0.246

N.B. Seeds were soaked for 12h and then treated with rhizobium inoculum
Plants were sprayed at 90d
A uniform basal dose of 45kg N, 30kg P and 30kg K/ha was applied
N.S. Non-significant.

4.3.5.2 Pod length

S_3+F_W gave maximum value for pod length (Table 24). However, it was equal to those for S_W+F_2, S_2+F_3 and S_3+F_1. The increase due to S_3+F_W was 5.04% compared with S_W+F_W. On the other hand, S_4+F_3 gave the lowest value for this parameter.

4.3.5.3 Seed number/pod

For this parameter, S_3+F_W again proved significantly optimum among all treatments (Table 24). This treatment exhibited 9.49% increase in seed number over S_W+F_W. Treatment S_4+F_3 gave lowest value which differed critically from those for the remaining treatments, except S_W+F_W.

4.3.5.4 1,000 seed weight

This parameter was not significantly affected by pyridoxine treatments (Table 24).

4.3.5.5 Seed yield

Seed yield was significantly maximum in S_3+F_W (Table 24). This treatment enhanced seed yield by 31.80% in comparison with S_W+F_W. Among all treatments, S_W+F_W produced significantly lowest seed yield.

4.3.6 Seed protein

Seed protein content was significantly affected by all pyridoxine treatments (Table 24). Of these, S_3+F_W exhibited significantly highest protein content in seeds. The increase due to this treatment was 2.44% in comparison with S_W+F_W. Treatment S_4+F_3 gave significantly lowest value for this parameter.

4.4 Experiment 4

In this simple randomised field trial, the effect of pre-sowing seed treatment with graded pyridoxine solution was studied on growth parameters, net assimilation rate, leaf nitrate reductase activity, leaf NPK content, seed yield and seed protein content of summer moong var. K-851. The treatments comprised S_W (water-soaked), S_1(0.1%), S_2(0.2%), S_3(0.3%), S_4(0.4%) and S_5(0.5% pyridoxine solution). The data are summarised in Tables 25-30 and described below.

4.4.1 Growth characteristics

Six growth parameters, namely plant length, root length, root nodule number, leaf number, fresh weight and dry weight, were studied at 20, 30, 40 and 50d after sowing. Root nodule number was recorded at 30, 40 and 50d because they were not conspicuous at 20d. All parameters, except fresh and dry weight as well as

leaf number at 20d, were significantly affected by pyridoxine treatments (Table 25-27).

4.4.1.1 Plant length

Plant length was significantly affected by various treatments at all four stages (Table 25). At 20d, S_1 produced significantly tallest plants. The value obtained in this treatment was 14.03% more than that in S_W. Treatment S_4 gave the lowest value for this parameter which differed critically from those for all other treatments, except S_W. On the other hand, S_3 gave significantly highest value for plant length at the later three stages, but at 40d its effect was at par with those of S_1 and S_2. Treatment S_3 enhanced plant length by 20.38%, 7.69% and 9.62% at 30, 40 and 50d respectively in comparison with S_W. At 30 and 50d, S_5 produced shortest plants and the value differed significantly from those in all other treatments. At 40d also, S_5 gave the lowest value but was at par with S_W.

4.4.1.2 Root length/plant

At 20d, root length was found significantly maximum in S_2 and the value was 12.89% more than that in S_W (Table 25). At 30d, S_3 proved significantly optimum for root length and this treatment enhanced it by 36.72% compared with S_W. However, at 40 and 50d, root length was maximum in S_4. The values obtained in

Table 25. Effect of pre-sowing seed treatment with pyridoxine on plant length and root length of summer moong var. K-851 (Mean of three replicates).

Treatments	Plant length (cm)				Root length/plant (cm)			
	Days after sowing							
	20	30	40	50	20	30	40	50
S_W	16.250	28.610	40.440	45.00	6.050	8.470	6.940	6.130
S_1	18.530	29.390	43.150	44.890	6.220	9.440	7.330	6.500
S_2	17.390	30.000	43.550	45.890	6.830	9.600	7.720	7.110
S_3	17.170	34.440	43.550	49.330	6.380	11.580	7.830	7.330
S_4	16.160	29.720	39.800	42.670	6.390	10.040	8.340	7.670
S_5	17.210	26.580	41.310	40.330	5.830	9.870	7.800	7.280
C.D. at 5%	0.821	0.926	1.270	0.944	0.273	0.841	0.755	0.943

N.B. Seeds were soaked for 4h and then treated with rhizobium inoculum

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

this treatment were at par with those for S_2, S_3 and S_5 at both these stages. The increase due to S_4 was noted to be 20.17% and 25.12% at 40 and 50d respectively in comparison with S_W. At 20d, S_5 gave the lowest value for root length which differed critically from those for all other treatments, except S_W. However, at 30, 40 and 50d, S_W gave the lowest values for this parameter which differed significantly from all other treatments, except S_1 at 40 and 50d.

4.4.1.3 Root nodule number/plant

Root nodules were not conspicuous at 20d (Table 26). Therefore, they were counted at 30, 40 and 50d only and were found to be significantly affected by pyridoxine. Among various treatments, S_3 produced significantly optimum number of root nodules at 30 and 40d. This treatment gave maximum number of nodules at 50d also but the value was equal to those for S_1, S_2 and S_4. Treatment S_3 increased root nodules by 144.00%, 25.12% and 25.30% at 30, 40 and 50d respectively in comparison with S_W. Further, S_W gave minimum root nodules at 30 and 50d. However, the values were at par with those for S_1 at 30d and with S_1, S_4 and S_5 at 50d. At 40d, S_5 gave the lowest value for this parameter but its effect was equal to those of S_W, S_1 and S_4.

Table 26. Effect of pre-sowing seed treatment with pyridoxine on root nodule number and leaf number of summer moong var. K-851 (Mean of three replicates).

Treatments	Root nodule number/plant				Leaf number/plant			
	Days after sowing							
	20	30	40	50	20	30	40	50
S_W	-	8.000	10.470	3.280	8.000	11.000	14.330	13.330
S_1	-	8.880	10.990	3.970	7.900	11.550	16.550	14.440
S_2	-	12.480	11.560	4.080	6.770	11.000	16.550	16.550
S_3	-	19.520	13.100	4.110	7.300	13.000	17.520	16.550
S_4	-	16.000	10.320	3.470	6.700	11.750	15.890	15.890
S_5	-	11.520	9.950	3.320	6.900	12.220	15.780	15.780
C.D. at 5%	-	2.054	0.737	0.669	N.S.	1.248	1.108	0.911

N.B. Seeds were soaked for 4h and then treated with rhizobium inoculum

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

N.S. Non-significant

4.4.1.4 Leaf number/plant

Leaf number was significantly enhanced by pyridoxine treatments at all stages, except at 20d (Table 26). Treatment S_3 exhibited maximum number of leaves at 30, 40 and 50d. However, its effect was at par with those of S_5 at 30d, with S_1 and S_2 at 40d and with S_2, S_4 and S_5 at 50d. Treatment S_3 increased leaf number by 18.18%, 22.26% and 24.16% at 30, 40 and 50d respectively over S_W. Treatment S_W produced significantly minimum number of leaves at all stages, except at 30d where its effect was equalled by S_1, S_2, S_4 and S_5.

4.4.1.5 Fresh weight/plant

Fresh weight was significantly affected by pyridoxine at 30, 40 and 50d only (Table 27). Treatment S_3 exhibited maximum fresh weight at each of these stages. However, the value was at par with those for S_4 and S_5 at 30d, S_2 at 40d and S_1 and S_2 at 50d. Treatment S_3 enhanced fresh weight by 27.76%, 66.75% and 18.39% at 30, 40 and 50d respectively in comparison with S_W. At 30d, S_2 gave the lowest value for this parameter but was equal to S_1 and S_W. At 40d, S_W gave significantly lowest value; but at 50d, S_5 showed critically lowest value than those for all other treatments, except S_4.

Table 27. Effect of pre-sowing seed treatment with pyridoxine of fresh weight and dry weight of summer moong var. K-851 (Mean of three replicates).

Treatments	Fresh weight/plant (g)				Dry weight/plant (g)			
	Days after sowing							
	20	30	40	50	20	30	40	50
S_W	1.130	3.890	8.330	15.060	0.153	0.548	2.880	3.560
S_1	1.370	3.520	12.330	17.000	0.147	0.520	3.110	3.920
S_2	1.220	3.330	13.220	17.000	0.147	0.518	3.100	3.920
S_3	1.110	4.970	13.890	17.830	0.151	0.681	4.320	5.340
S_4	1.020	4.510	10.670	14.440	0.148	0.677	2.780	3.590
S_5	1.220	4.190	10.330	13.330	0.149	0.616	2.380	2.780
C.D. at 5%	N.S.	0.828	1.268	1.336	N.S.	0.050	0.824	0.940

N.B. Seeds were soaked for 4h and then treated with rhizobium inoculum

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

N.S. Non-significant

4.4.1.6 Dry weight/plant

Seed treatment with pyridoxine did not affect dry weight of plants significantly at 20d, but the effect was significant at the remaining stages. At 30d, maximum dry weight was recorded in S_3; but the value was at par with that for S_4. Treatment S_3 increased dry weight by 24.27% in comparison with S_W. Lowest value was recorded in S_2 and was statistically equal to those for S_W and S_1. At 40 and 50d, S_3 exhibited significantly highest dry weight. The increase due to this treatment was 50.00% at both 40 and 50d in comparison with S_W. The highest concentration of pyridoxine, i.e. 0.5% (S_5), gave the lowest dry weight at 40d but the value did not show significant difference from those for S_W, S_1, S_2 and S_4. At 50d also, S_5 exhibited lowest dry weight but was at par with S_W and S_4 (Table 27).

4.4.2 Net assimilation rate (NAR)

NAR was estimated for the 20-30d, 30-40d and 40-50d periods (Table 28) and was found to be significantly affected by pyridoxine treatments. During 20-30d, S_4 gave significantly optimum value for NAR, showing an increase of 28.16% in comparison with S_W. Lowest value for this parameter was recorded in S_1; but it did not show significant difference from S_W. During 30-40d and 40-50d, S_3 gave significantly highest NAR and enhanced it by 50.21% and 49.75% respectively in comparison with S_W. The highest

Table 28. Effect of pre-sowing seed treatment with pyridoxine on net assimilation rate (NAR) and nitrate reductase activity (NRA) of summer moong var. K-851 (Mean of three replicates).

Treatments	NAR ($x10^{-4}$ g/cm^2/d)			NRA (n mol NO_2^-/g/h)			
	Days interval			Days after sowing			
	20-30	30-40	40-50	20	30	40	50
S_W	8.040	14.312	2.247	133.980	111.650	120.110	110.050
S_1	7.640	13.767	2.322	148.330	110.850	119.600	110.050
S_2	8.561	17.187	2.739	163.490	114.840	133.710	118.650
S_3	9.562	21.498	3.365	173.830	119.620	134.240	127.170
S_4	10.304	13.249	2.859	153.920	106.860	115.800	109.600
S_5	8.627	10.482	1.438	148.330	107.660	110.100	107.660
C.D. at 5%	0.523	0.591	0.111	8.601	2.054	5.524	5.484

N.B. Seeds were soaked for 4h and then treated with rhizobium inoculum

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

concentration of pyridoxine treatment, i.e. 0.5% (S_5), resulted in significantly lowest NAR at both these periods.

4.4.3 Nitrate reductase activity (NRA)

NRA was measured in leaves at 20, 30, 40 and 50d (Table 28). Pyridoxine treatments significantly affected NRA levels in leaves at all stages. Among different treatments, S_3 gave significantly optimum enzyme activity at each of these stages. The increase due to this treatment at 20, 30, 40 and 50d over S_W was 29.74%, 7.14%, 11.76% and 15.56% respectively. Significant lowest value for NRA at 20d was obtained in S_W. At 30d, lowest value for NRA was obtained in S_4; but it was equal to that for S_5. On the other hand, S_5 at 40d resulted in significantly minimum enzyme activity. At 50d also, S_5 gave the lowest value for NRA but did not differ critically from S_2 and S_3.

4.4.4 Leaf NPK content

Leaf NPK content, estimated at 20, 30, 40 and 50d, was significantly affected by pyridoxine treatments, except leaf potassium at 50d. The data are summarised in Table 29 and given below.

4.4.4.1 Nitrogen

Nitrogen content in leaves was noted to be highest in S_3 at all stages (Table 29). The value recorded for this treatment differed significantly from those for other treatments at all stages, except at 20 and 50d where its effect was equal to that of S_2. The increase due to S_3 over S_W was 2.15%, 1.87%, 2.30% and 1.00% at 20, 30, 40 and 50d respectively. On the other hand, S_5 gave significantly lowest value for leaf nitrogen content at all stages, except at 20 and 50d where it proved at par with S_4.

4.4.4.2 Phosphorus

Leaf phosphorus content was found significantly optimum in S_3 at all stages, except at 30d where S_2 gave significantly maximum phosphorus content (Table 29). However, at this stage, the value recorded in S_3 was next to S_2. Treatment S_3 enhanced leaf phosphorus by 0.01%, 0.06%, 0.11% and 0.12% at 20, 30, 40 and 50d respectively in comparison with S_W. The lowest phosphorus content was noted in S_5 at 20, 40 and 50d. The value given by this treatment significantly differed from those for all other treatments at 20 and 50d but at 40d, it was at par with that for S_5. At 30d, S_W showed poorest effect which was significantly different from that of all other treatments, except S_1 and S_5.

Table 29. Effect of pre-sowing seed treatment with pyridoxine on leaf NPK content of summer moong var. K-851 (Mean of three replicates).

Treatments	Nitrogen (%)				Phosphorus (%)				Potassium (%)			
	Days after sowing											
	20	30	40	50	20	30	40	50	20	30	40	50
S_W	5.250	5.130	4.000	2.500	0.472	0.414	0.344	0.254	4.633	3.333	2.200	1.896
S_1	5.950	5.600	4.500	2.650	0.528	0.418	0.400	0.280	5.021	3.600	2.224	2.004
S_2	6.950	5.880	5.000	3.500	0.544	0.496	0.416	0.288	5.450	3.675	2.459	2.113
S_3	7.400	7.000	6.300	3.500	0.568	0.472	0.451	0.372	6.316	4.248	2.896	2.017
S_4	4.250	3.570	3.220	1.400	0.496	0.464	0.368	0.208	5.499	3.961	2.400	2.000
S_5	3.700	2.500	2.420	1.500	0.448	0.432	0.344	0.160	5.269	3.600	2.116	1.953
C.D. at 5%	0.750	0.829	0.506	0.330	0.020	0.021	0.023	0.030	0.277	0.232	0.167	N.S.

N.B. Seeds were soaked for 4h and then treated with rhizobium inoculum

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

N.S. Non-significant

4.4.4.3 Potassium

The effect of pyridoxine application was significant on the potassium content of leaves at 20, 30 and 40d only (Table 29). Among different treatments, S_3 exhibited significantly higher values in comparison with those for all other treatments at each of these stages. The increase due to this treatment over S_W was 1.68%, 0.91% and 0.70% at 20, 30 and 40d respectively. At 20 and 30d, S_W gave significantly lowest value for this parameter, while at 40d, S_5 resulted in minimum leaf potassium content. However, the value obtained in this treatment was equal to those in S_W and S_1.

4.4.5 Yield characteristics

Five yield characters, namely pod number/plant, pod length, seed number/pod, 1,000 seed weight and seed yield, were studied at harvest. All these parameters were significantly affected by seed treatment with pyridoxine. The data are summarised in Table 30 and described below.

4.4.5.1 Pod number/plant

As is clear from Table 30, the maximum number of pods was observed in S_3. However, the value differed significantly from those for S_W and S_1 only. S_3 enhanced pod number by 45.02%

Table 30. Effect of pre-sowing seed treatment with pyridoxine on yield parameters and seed protein content of summer moong var. K-851 (Mean of three replicates).

Treatments	Pod number/ plant	Pod length (cm)	Seed number/ pod	1,000 seed weight (g)	Seed yield (q/ha)	Protein content (%)
S_W	8.440	6.890	7.270	40.190	8.920	24.500
S_1	9.780	6.940	7.860	39.450	8.660	24.500
S_2	10.660	7.070	7.800	38.032	10.400	25.780
S_3	12.240	8.190	10.370	37.690	13.820	25.070
S_4	11.340	7.530	9.260	37.430	11.560	23.660
S_5	11.120	7.260	8.930	34.130	9.020	23.330
C.D. at 5%	1.623	0.398	0.552	0.707	1.540	0.696

N.B. Seeds were soaked for 4h and then treated with rhizobium inoculum

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

in comparison with S_W. The lowest number of pods was noted in S_W but it was equalled by S_1.

4.4.5.2 Pod length

Optimum pod length was recorded in S_3 and the value differed critically from those obtained in the remaining treatments. The increase due to S_3 was 18.87% compared with S_W. Treatment S_W gave minimum pod length, but the value did not differ from those for S_1, S_2 and S_5 significantly (Table 30).

4.4.5.3 Seed number/pod

Seed number was also significantly optimum in S_3. This treatment enhanced seed number by 42.64% in comparison with S_W in which lowest seed number was recorded. However, the value obtained in S_W did not differ from that in S_2 significantly (Table 30).

4.4.5.4 1,000 seed weight

Heaviest seeds were found in S_W and all the pyridoxine treatments gave significantly lower values for this parameter in comparison with S_W (Table 30). A decrease of 6.22% in seed weight was noted in S_3. Significantly lightest seeds were produced by S_5.

4.4.5.5 Seed yield

Seed yield was significantly maximum in S_3. The treatment enhanced seed yield by 54.93% in comparison with S_W. Treatment S_1 gave the lowest seed yield but was equalled by S_W and S_5 in its effect (Table 30).

4.4.6 Seed protein

Seed protein content was also affected significantly by pyridoxine treatment (Table 30). Significantly optimum seed protein content was noted in S_2, being 1.28% more than in S_W. The highest concentration of pyridoxine solution, i.e. 0.5% (S_5), resulted in lowest protein content in seeds. However, its effect was equal to that of S_4.

4.5 Experiment 5

In this simple randomised field experiment on summer moong var. K-851, the treatments consisted of foliar spray at 35 or 45d of 0.0%, 0.025%, 0.05%, 0.1% and 0.2% aqueous pyridoxine solution and were designated as $F_{W(35)}$, $F_{1(35)}$, $F_{2(35)}$, $F_{3(35)}$, $F_{4(35)}$, $F_{W(45)}$, $F_{1(45)}$, $F_{2(45)}$, $F_{3(45)}$ and $F_{4(45)}$ respectively.

The same parameters as in Experiment 4 were studied. Both controls, i.e. $F_{W(35)}$ and $F_{W(45)}$ proved at par with each other for all parameters. Therefore, the values obtained in the optimum

treatment were compared with the respective water sprayed control only. The data are presented in Tables 31-36 and described below.

4.5.1 Growth characteristics

Five growth parameters (plant length, root length, leaf number, fresh weight and dry weight) were studied at 45 and 55d after sowing. A sixth character, root nodule number was observed at 45d only as the nodules degenerated at the later stage. All these parameters, except root length at 55d, were significantly affected by pyridoxine spray. The data are given in Tables 31-33 and described below.

4.5.1.1 Plant length

Among various pyridoxine spray treatments, $F_{3(35)}$ produced significantly tallest plants at 45 and 55d (Table 31). This treatment increased plant length by 17.07% at 45d and 17.53% at 55d over $F_{W(35)}$. At 45d, $F_{W(45)}$ produced shortest plants; but the value was at par with those for $F_{W(35)}$ and $F_{1(35)}$ while at 55d, $F_{4(45)}$ gave significantly lowest value.

4.5.1.2 Root length/plant

Root length was significantly affected only at 45d by pyridoxine spray treatments (Table 31). Among these treatments,

Table 31. Effect of pyridoxine spray on plant length and root length of summer moong var. K-851 (Mean of three replicates).

Treatments	Plant length (cm)		Root length/plant (cm)	
	Days after sowing			
	45	55	45	55
$F_{W(35)}$	41.322	45.000	8.444	8.500
$F_{1(35)}$	41.089	47.000	8.555	9.778
$F_{2(35)}$	42.556	47.889	8.833	10.278
$F_{3(35)}$	48.378	52.889	8.944	11.333
$F_{4(35)}$	46.555	43.000	8.111	8.722
$F_{W(45)}$	40.000	45.167	8.356	9.500
$F_{1(45)}$	-	46.556	-	9.833
$F_{2(45)}$	-	47.556	-	9.172
$F_{3(45)}$	-	50.000	-	10.167
$F_{4(45)}$	-	41.222	-	9.056
C.D. at 5%	1.498	1.691	0.120	N.S.

N.B. Seeds were treated with rhizobium inoculum

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

N.S. Non-significant

$F_{3(35)}$ gave maximum length but the value was at par with that for $F_{2(35)}$. Treatment $F_{3(35)}$ increased root length by 5.92% in comparison with $F_{W(35)}$. Treatment $F_{4(35)}$ gave significantly lowest root length.

4.5.1.3 Root nodule number/plant

Root nodules were counted only at 45d as they degenerated at 55d (Table 32). Among various spray treatments, $F_{3(35)}$ produced maximum root nodules. However, its effect on root nodule number was at par with that of $F_{2(35)}$. Treatment $F_{3(35)}$ enhanced root nodule number by 36.22% compared with $F_{W(35)}$. Treatment $F_{W(45)}$ gave the lowest number of root nodules; but the value was at par with those for $F_{W(35)}$ and $F_{1(35)}$.

4.5.1.4 Leaf number/plant

$F_{3(35)}$ produced maximum leaves at both stages (Table 32). However, the value obtained in $F_{3(35)}$ was equal to that for $F_{4(35)}$ at 45d and to those for $F_{3(45)}$ and $F_{4(45)}$ at 55d. The increase due to $F_{3(35)}$ was 37.69% at 45d and 11.36% at 55d in comparison with $F_{W(35)}$. At the two stages, $F_{W(35)}$ and $F_{W(45)}$ gave significantly lowest number of leaves respectively.

Table 32. Effect of pyridoxine spray on root nodule number and leaf number of summer moong var. K-851 (Mean of three replicates).

Treatments	Root nodule number/plant		Leaf number/plant	
	Days after sowing			
	45	55	45	55
$F_{W(35)}$	6.682	-	12.750	23.647
$F_{1(35)}$	6.660	-	14.667	24.161
$F_{2(35)}$	8.889	-	15.750	25.467
$F_{3(35)}$	9.102	-	17.556	26.333
$F_{4(35)}$	7.109	-	17.000	25.125
$F_{W(45)}$	6.555	-	13.556	23.444
$F_{1(45)}$	-	-	-	25.451
$F_{2(45)}$	-	-	-	26.333
$F_{3(45)}$	-	-	-	26.000
$F_{4(45)}$	-	-	-	24.333
C.D. at 5%	0.395	-	1.009	0.343

N.B. Seeds were treated with rhizobium inoculum

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

4.5.1.5 Fresh weight/plant

$F_{3(35)}$ produced significantly maximum fresh weight at 45 and 55d (Table 33). This treatment enhanced the fresh weight by 86.75% at 45d and 39.77% at 55d in comparison with $F_{W(35)}$. However at 45d, both the controls, i.e. $F_{W(35)}$ and $F_{W(45)}$, exhibited significantly lowest values for this parameter. At 55d, $F_{4(45)}$ gave the lowest value which was at par with that for $F_{W(45)}$.

4.5.1.6 Dry weight/plant

At both samplings, $F_{3(35)}$ proved significantly optimum for dry weight (Table 33). The increase due to $F_{3(35)}$ over $F_{W(35)}$ was 53.83% at 45d and 65.42% at 55d. At 45d, $F_{W(45)}$ gave lowest value for this parameter which was equal to those for $F_{W(35)}$ and $F_{1(35)}$. At 55d, $F_{W(45)}$ produced the lowest dry weight, but the value did not differ significantly from those for $F_{W(35)}$ and $F_{4(45)}$.

4.5.2 Net assimilation rate (NAR)

NAR was computed for the 35-45d and 45-55d periods (Table 34). For both durations, $F_{3(35)}$ proved significantly optimum for NAR. $F_{3(35)}$ increased this parameter by 33.94% during the first period (35-45d) and 30.71% during the second period (45-55d) in comparison with $F_{W(35)}$. Treatment $F_{1(35)}$ gave the

Table 33. Effect of pyridoxine spray on fresh weight and dry weight of summer moong var. K-851 (Mean of three replicates).

Treatments	Fresh weight/plant(g)		Dry weight/plant(g)	
	Days after sowing			
	45	55	45	55
$F_{W(35)}$	7.556	17.600	2.049	3.045
$F_{1(35)}$	8.556	18.600	2.044	3.160
$F_{2(35)}$	8.444	22.100	2.690	4.243
$F_{3(35)}$	14.111	24.600	3.152	5.037
$F_{4(35)}$	12.222	20.000	3.000	3.896
$F_{W(45)}$	7.111	16.111	2.043	3.002
$F_{1(45)}$	-	18.600	-	3.144
$F_{2(45)}$	-	19.100	-	3.260
$F_{3(45)}$	-	20.700	-	3.458
$F_{4(45)}$	-	15.778	-	3.028
C.D. at 5%	0.476	1.645	0.076	0.130

N.B. Seeds were treated with rhizobium inoculum

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

Table 34. Effect of pyridoxine spray on net assimilation rate (NAR) and nitrate reductose activity (NRA) of summer moong var. K-851 (Mean of three replicate).

Treatments	NAR ($x10^{-4}$ g/cm^2/d)		NRA (n mol NO_2^-/g/h)	
	Days interval		Days after sowing	
	35–45	45–55	45	55
$F_{W(35)}$	9.934	3.217	111.614	102.792
$F_{1(35)}$	9.633	3.518	130.736	114.792
$F_{2(35)}$	12.370	3.947	153.056	121.170
$F_{3(35)}$	13.306	4.205	165.810	146.678
$F_{4(35)}$	11.839	2.023	154.491	140.620
$F_{W(45)}$	9.894	3.121	108.414	100.705
$F_{1(45)}$	-	3.365	-	124.358
$F_{2(45)}$	-	3.761	-	124.358
$F_{3(45)}$	-	3.797	-	136.678
$F_{4(45)}$	-	3.277	-	130.736
C.D. at 5%	0.532	0.166	4.758	3.960

N.B. Seeds were treated with rhizobium inoculum

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

lcwest value for NAR during the first period. However, its effect was at par with those of $F_{W(35)}$ and $F_{W(45)}$. During the second period, $F_{4(35)}$ gave significantly lowest NAR.

4.5.3 Nitrate reductase activity (NRA)

Leaf NRA was measured at 45 and 55d after sowing and was noted to be significantly affected by pyridoxine spray (Table 34). Among various spray treatments, $F_{3(35)}$ exhibited significantly maximum enzyme activity at both stages. The enzyme activity due to this treatment was enhanced by 48.56% at 45d and 42.69% at 55d compared with $F_{W(35)}$. At both stages, $F_{W(35)}$ and $F_{W(45)}$ gave significantly lowest enzyme activity.

4.5.4 Leaf NPK content

Leaf NPK content was estimated at 45 and 55d and was found to be significantly affected by pyridoxine spray. The data are summarised in Table 35 and described below.

4.5.4.1 Nitrogen

At 45d, $F_{3(35)}$ gave maximum leaf nitrogen content (Table 35). The value differed critically from those for the remaining treatments, except $F_{2(35)}$. Treatment $F_{3(35)}$ enhanced leaf nitrogen by 1.45% in comparison with $F_{W(35)}$. Treatment $F_{4(35)}$

Table 35. Effect of pyridoxine spray on leaf NPK content of summer moong var. K-851 (Mean of three replicates).

Treatments	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
	Days after sowing					
	45	55	45	55	45	55
$F_{W(35)}$	3.950	2.150	0.368	0.240	2.333	1.944
$F_{1(35)}$	4.200	2.300	0.384	0.272	2.000	2.031
$F_{2(35)}$	5.300	2.500	0.400	0.294	2.800	2.500
$F_{3(35)}$	5.400	3.950	0.560	0.331	3.043	2.692
$F_{4(35)}$	2.900	1.750	0.384	0.204	2.167	1.912
$F_{W(45)}$	3.750	2.170	0.352	0.226	2.258	1.978
$F_{1(45)}$	-	2.450	-	0.288	-	2.000
$F_{2(45)}$	-	2.850	-	0.296	-	2.190
$F_{3(45)}$	-	2.500	-	0.343	-	2.461
$F_{4(45)}$	-	1.950	-	0.224	-	1.939
C.D. at 5%	0.484	0.521	0.030	0.032	0.338	0.152

N.B. Seeds were treated with rhizobium inoculum

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

exhibited significantly lowest leaf nitrogen content. At 55d, $F_{3(35)}$ resulted in significantly optimum leaf nitrogen content. The value obtained in this treatment was 1.80% higher than that for $F_{W(35)}$. Lowest value was recorded in $F_{4(35)}$; but its effect was at par with those of $F_{W(35)}$, $F_{W(45)}$ and $F_{4(35)}$.

4.5.4.2 Phosphorus

At 45d, $F_{3(35)}$ exhibited significantly optimum leaf phosphorus content (Table 35), enhancing it by 0.19% in comparison with $F_{W(35)}$. Treatment $F_{W(45)}$ gave the lowest value for this parameter; but its effect was at par with that of $F_{W(35)}$. At 55d, $F_{3(45)}$ resulted in maximum leaf phosphorus content and differed critically from the remaining treatments, except $F_{3(35)}$. The increase due to $F_{3(45)}$ over $F_{W(45)}$ was 0.12%. Treatment $F_{4(35)}$ gave the lowest value which was statistically equal to those for $F_{W(45)}$ and $F_{4(45)}$.

4.5.4.3 Potassium

At 45d, potassium content in leaves (Table 35) was optimum in $F_{3(35)}$. However, its effect was equalled by that of $F_{2(35)}$. Treatment $F_{3(35)}$ increased the leaf potassium content by 0.71% in comparison with $F_{W(35)}$. Treatment $F_{1(35)}$ gave the lowest value which did not differ significantly from those for $F_{W(35)}$, $F_{W(45)}$ and $F_{4(35)}$. At 55d, $F_{3(35)}$ manifested significantly highest

value for leaf potassium. This treatment enhanced the potassium content by 0.75% compared with $F_{W(35)}$. Treatment $F_{4(35)}$ gave the lowest value for this parameter; but its effect was equal to those of $F_{W(35)}$, $F_{W(45)}$, $F_{1(35)}$, $F_{1(45)}$ and $F_{4(45)}$.

4.5.5 Yield characteristics

Five yield characters, namely pod number/plant, pod length, seed number/pod, 1,000 seed weight and seed yield, were studied at harvest. All these parameters were significantly affected by pyridoxine spray (Table 36). The details are considered below.

4.5.5.1 Pod number/plant

$F_{3(35)}$ produced significantly maximum number of pods (Table 36). The increase due to $F_{3(35)}$ over $F_{W(35)}$ was 40.43%. Treatment $F_{4(45)}$ produced minimum number of pods; but the value was at par with that for $F_{4(35)}$.

4.5.5.2 Pod length

$F_{3(35)}$ gave maximum pod length (Table 36) and the value differed critically from those for the remaining treatments, except $F_{3(45)}$. Treatment $F_{3(35)}$ increased pod length by 6.44% compared with $F_{W(35)}$. Treatment $F_{4(45)}$ produced pods of minimum length but the value was at par with those for $F_{W(35)}$, $F_{W(45)}$ and $F_{4(35)}$.

Table 36. Effect of pyridoxine spray on yield parameters and seed protein content of summer moong var. K-851 (Mean of three replicates).

Treatments	Pod number/ plant	Pod length (cm)	Seed number/ pod	1,000 seed weight (g)	Seed yield (q/ha)	Protein content (%)
$F_{W(35)}$	8.078	7.171	8.133	40.670	8.810	23.340
$F_{1(35)}$	9.350	7.300	8.800	39.800	9.336	24.360
$F_{2(35)}$	10.151	7.413	9.154	39.807	9.346	24.990
$F_{3(35)}$	11.344	7.633	9.312	41.490	11.184	26.320
$F_{4(35)}$	7.573	7.107	7.867	40.327	9.830	24.120
$F_{W(45)}$	8.014	7.119	8.115	40.006	8.804	23.110
$F_{1(45)}$	8.976	7.280	8.867	42.990	9.342	24.000
$F_{2(45)}$	9.537	7.346	8.875	40.387	9.740	25.460
$F_{3(45)}$	10.624	7.487	9.167	40.620	10.799	25.600
$F_{4(45)}$	7.550	7.021	7.928	40.950	9.308	23.320
C.D. at 5%	0.192	0.195	0.173	1.018	0.185	0.545

N.B. Seeds were treated with rhizobium inoculum

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

4.5.5.3 Seed number/pod

Maximum seed number (Table 36) was obtained in $F_{3(35)}$; but its effect was equalled by those of $F_{2(35)}$ and $F_{3(45)}$. Treatment $F_{3(35)}$ produced 14.50% more seeds than did $F_{W(35)}$. Treatment $F_{4(35)}$, giving lowest value for this parameter, differed critically from the remaining treatments, except $F_{4(45)}$.

4.5.5.4 1,000 seed weight

Unlike the other yield parameters, significantly heaviest seeds (Table 36) were found in $F_{1(45)}$ which increased 1,000 seed weight by 7.46% over $F_{W(45)}$. Treatment $F_{1(35)}$ produced lightest seeds; but the value was at par with those for $F_{W(35)}$, $F_{W(45)}$, $F_{2(35)}$, $F_{4(35)}$, $F_{2(45)}$ and $F_{3(45)}$.

4.5.5.5 Seed yield

Treatment $F_{3(35)}$ resulted in significantly optimum seed yield (Table 36) which was 26.95% higher than that in $F_{W(35)}$. Both the controls, i.e. $F_{W(35)}$ and $F_{W(45)}$, gave significantly lowest seed yield.

4.5.6 Seed protein

Protein content estimated in seeds at harvest was found to be significantly affected by pyridoxine spray (Table 36). Among

various spray treatments, $F_{3(35)}$ proved significantly optimum for this parameter. The increase due to this treatment was 2.98% in comparison with $F_{W(35)}$. Treatment $F_{W(45)}$ produced minimum seed protein; but the value was at par with those for $F_{W(35)}$ and $F_{4(45)}$.

4.6 Experiment 6

In this simple randomised field trial on summer moong var. K-851, the combined treatments consisted of soaking the seeds for 4h in 0.0% and 0.3% and foliar spray at 35 or 45d of 0.0%, 0.1%, 0.2% and 0.3% aqueous pyridoxine solution. These were designated as $S_W+F_{W(35)}$, $S+F_{W(35)}$, $S+F_{1(35)}$, $S+F_{2(35)}$, $S+F_{3(35)}$, $S_W+F_{W(45)}$, $S+F_{W(45)}$, $S+F_{1(45)}$, $S+F_{2(45)}$ and $S+F_{3(45)}$.

The same parameters as in Experiments 4 and 5 were studied. Both the controls, i.e. $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ proved at par with each other for all parameters. Therefore, the values obtained in the optimum treatment were compared with the respective control only. The data are condensed in Tables 37-42 and described below.

4.6.1 Growth characteristics

Five growth characteristics, namely plant length, root length, leaf number, fresh weight and dry weight were studied at 45 and 55d after sowing. However, root nodules could be counted only at 45d after sowing because they degenerated at 55d. All these parameters were significantly affected by the combined pyridoxine soaking and spray treatments (Tables 37-39).

4.6.1.1 Plant length

At 45d, $S+F_{1(35)}$ produced significantly tallest plants (Table 37). This treatment increased plant length by 23.38% in comparison with $S_W+F_{W(35)}$. The values obtained in $S+F_{W(35)}$ and $S+F_{W(45)}$ being next to $S+F_1$, were at par with each other. At 55d, $S+F_{W(35)}$ and $S+F_{W(45)}$, showing equal effect, resulted in significantly tallest plants. The value given by $S+F_{W(35)}$ was 15.58% and by $S+F_{W(45)}$, 19.30% higher than those for $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively, which gave significantly lowest values at both stages.

4.6.1.2 Root length/plant

At 45d, $S+F_{W(35)}$, $S+F_{W(45)}$ and $S+F_{1(35)}$, being at par with each other, produced maximum root length (Table 37). The increase due to $S+F_{W(35)}$ and $S+F_{W(45)}$ was 16.65% and 16.93% in comparison with $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively. Both the controls, i.e. $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ exhibited significantly lowest values. At 55d also, $S+F_{W(35)}$ and $S+F_{W(45)}$ proved optimum but their effect was at par with those of $S+F_{1(35)}$ and $S+F_{2(45)}$. The treatments $S+F_{W(35)}$ and $S+F_{W(45)}$ enhanced root length by 15.38% and 21.39% over $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively. Treatment $S+F_{2(45)}$, giving lowest value for this parameter, was equal in its effect to $S+F_{3(45)}$.

Table 37. Combined effect of pyridoxine soaking and spray on plant length and root length of summer moong var. K-851 (Mean of three replicates).

Treatments	Plant length (cm)		Root length/plant (cm)	
	Days after sowing			
	45	55	45	55
$S_W+F_{W(35)}$	41.244	49.222	7.667	8.667
$S+F_{W(35)}$	50.333	56.889	8.944	10.000
$S+F_{1(35)}$	50.889	53.178	8.944	9.222
$S+F_{2(35)}$	48.800	53.222	8.389	9.222
$S+F_{3(35)}$	46.378	51.667	8.389	8.778
$S_W+F_{W(45)}$	41.555	47.778	7.554	9.778
$S+F_{W(45)}$	50.333	57.000	8.833	8.055
$S+F_{1(45)}$	-	53.667	-	9.000
$S+F_{2(45)}$	-	53.333	-	7.555
$S+F_{3(45)}$	-	52.444	-	7.800
C.D. at 5%	0.462	1.905	0.225	0.803

N.B. Seeds were soaked for 4h and then treated with rhizobium inoculum

Plants were sprayed either at 35 or 45d

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

4.6.1.3 Root nodule number/plant

As mentioned earlier, root nodules were counted only at 45d (Table 38). Treatments $S+F_{W(35)}$ and $S+F_{W(45)}$, having equal effect on this parameter, gave significantly higher values in comparison with those for all other treatments. Root nodules were increased by 36.46% in $S+F_{W(35)}$ and by 46.07% in $S+F_{W(45)}$ compared with $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively. $S+F_{3(35)}$ produced significantly lowest number of root nodules.

4.6.1.4 Leaf number/plant

At 45d, $S+F_{2(35)}$ gave maximum value for leaf number (Table 38). However, its effect was at par with that of $S+F_{3(35)}$. Treatment $S+F_{2(35)}$ enhanced leaf number by 36.44% over $S_W+F_{W(35)}$. On the other hand, $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ showed lowest value for this parameter. At 55d, $S+F_{2(35)}$ again produced significantly optimum number of leaves, showing an increase of 26.56% compared with $S_W+F_{W(35)}$. On the other hand, $S+F_{3(45)}$ produced lowest number of leaves. However, the effect of this treatment was equal to those of $S+F_{3(35)}$, $S+F_{1(45)}$ and $S+F_{2(45)}$.

4.6.1.5 Fresh weight/plant

At 45d, $S+F_{W(35)}$ and $S+F_{W(45)}$, giving equal values, produced maximum fresh weight. The effect of these treatments was significantly more pronounced than those of the remaining

Table 38. Combined effect of pyridoxine soaking and spray on root nodule number and leaf number of summer moong var. K-851 (Mean of three replicates).

Treatments	Root nodule number/plant		Leaf number/plant	
	Days after sowing			
	45	55	45	55
$S_W+F_{W(35)}$	6.865	-	14.333	21.333
$S+F_{W(35)}$	9.368	-	15.889	22.000
$S+F_{1(35)}$	8.489	-	16.875	23.000
$S+F_{2(35)}$	7.668	-	19.556	27.000
$S+F_{3(35)}$	5.333	-	18.222	20.333
$S_W+F_{W(45)}$	6.542	-	15.556	21.667
$S+F_{W(45)}$	9.556	-	15.778	23.889
$S+F_{1(45)}$	-	-	-	20.667
$S+F_{2(45)}$	-	-	-	20.111
$S+F_{3(45)}$	-	-	-	19.333
C.D. at 5%	0.407	-	1.388	1.998

N.B. Seeds were soaked for 4h and then treated with rhizobium inoculum

Plants were sprayed either at 35 or 45d

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

treatments, except $S+F_{1(35)}$ and $S+F_{2(35)}$. The values obtained in $S+F_{W(35)}$ and $S+F_{W(45)}$ were 56.90% and 51.66% higher than those in $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively. Both these controls gave significantly the lowest value for this parameter. At 55d, $S+F_{1(35)}$ gave significantly highest fresh weight which was 56.61% more in comparison with $S_W+F_{W(35)}$. Treatments $S_W+F_{W(35)}$, $S_W+F_{W(45)}$ and $S+F_{3(35)}$ gave significantly lowest valuesfor this parameter, which were at par with each other (Table 39).

4.6.1.6 Dry weight/plant

At 45d, $S+F_{W(35)}$ and $S+F_{W(45)}$, having equal effect, gave maximum values for dry weight (Table 39). The values differed critically from those for all other treatments, except $S+F_{1(35)}$. The increase due to $S+F_{W(35)}$ was 69.88% and that due to $S+F_{W(45)}$, 65.10% in comparison with $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively. Moreover, both the controls,i.e. $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$, gave significantly lowest value for this parameter. At 55d, $S+F_{W(35)}$ and $S+F_{W(45)}$ again gave significantly maximum values for dry weight; but the latter was at par with $S+F_{1(45)}$. Treatment $S+F_{W(35)}$ exhibited 70.56% and $S+F_{W(45)}$, 65.25% more dry weight than $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively which gave significantly lowest values.

Table 39. Combined effect of pyridoxine soaking and spray on fresh weight and dry weight of summer moong var. K-851 (Mean of three replicates).

Treatments	Fresh weight/plant (g)		Dry weight/plant (g)	
	Days after sowing			
	45	55	45	55
$S_W+F_{W(35)}$	6.444	18.333	2.178	3.108
$S+F_{W(35)}$	10.111	26.000	3.700	5.301
$S+F_{1(35)}$	9.778	28.711	3.526	4.758
$S+F_{2(35)}$	9.556	20.778	3.199	4.610
$S+F_{3(35)}$	9.000	18.000	3.020	4.032
$S_W+F_{W(45)}$	6.667	18.000	2.189	3.134
$S+F_{W(45)}$	10.111	25.667	3.614	5.179
$S+F_{1(45)}$	-	22.111	-	4.971
$S+F_{2(45)}$	-	25.556	-	4.766
$S+F_{3(45)}$	-	21.222	-	4.110
C.D. at 5%	0.859	2.169	0.239	0.232

N.B. Seeds were soaked for 4h and then treated with rhizobium inoculum

Plants were sprayed either at 35 or 45d

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

4.6.2 Net assimilation rate (NAR)

NAR was computed for 35–45d and 45–55d periods (Table 40). Pyridoxine treatments significantly affected this parameter at both these periods. Treatments $S+F_{W(35)}$ and $S+F_{W(45)}$, exhibiting equal effect, gave significantly highest value for NAR during 35–45d. Treatment $S+F_{W(35)}$ enhanced NAR by 48.50% and $S+F_{W(45)}$, by 43.71% compared with $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively. Treatment $S+F_{3(35)}$ showed minimum value for NAR which differed critically from those for the remaining treatments, except both the controls. During 45–55d, $S+F_{W(35)}$, $S+F_{W(45)}$ and $S+F_{1(45)}$, having equal effect, gave significantly higher values for NAR than the remaining treatments. The value in $S+F_{W(35)}$ was 49.77% and for $S+F_{W(45)}$, 41.71% higher than those in $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively. Treatment $S+F_{3(45)}$ manifested significantly lowest value for this parameter.

4.6.3 Nitrate reductase activity (NRA)

NRA, measured in leaves at 45 and 55d, was found to be significantly affected by pyridoxine treatments (Table 40). Among various treatments, $S+F_{W(35)}$ and $S+F_{W(45)}$ exhibited significantly maximum enzyme activity at both stages of sampling. The enzyme activity due to $S+F_{W(35)}$ was increased by 30.56% and 38.42% at 45 and 55d respectively in comparison with $S_W+F_{W(35)}$, while at 45 and 55d, $S+F_{W(45)}$ showed 32.15% and 34.00% higher enzyme activity

Table 40. Combined effect of pyridoxine soaking and spray on net assimilation rate (NAR) and nitrate reductase activity (NRA) of summer moong var. K-851 (Mean of three replicates).

Treatments	NAR ($x10^{-4}$ g/cm^2/d)		NRA (n mol NO_2^-/g/ha)	
	Days interval		Days after sowing	
	35–45	45–55	45	55
$S_W+F_{W(35)}$	9.479	2.875	114.792	92.472
$S+F_{W(35)}$	14.076	4.306	149.868	128.000
$S+F_{1(35)}$	12.346	3.183	133.924	116.604
$S+F_{2(35)}$	9.779	3.216	127.546	108.414
$S+F_{3(35)}$	8.838	2.670	100.705	82.906
$S_W+F_{W(45)}$	9.370	2.781	111.604	92.805
$S+F_{W(45)}$	13.466	3.941	147.490	124.358
$S+F_{1(45)}$	-	4.053	-	108.412
$S+F_{2(45)}$	-	3.482	-	89.282
$S+F_{3(45)}$	-	1.604	-	79.716
C.D. at 5%	0.740	0.381	3.986	5.067

N.B. Seeds were soaked for 4h and then treated with rhizobium inoculum

Plants were sprayed either at 35 or 45d

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

respectively than $S_W+F_{W(45)}$. At 45d, $S+F_{3(35)}$ manifested significantly lowest enzyme activity in leaves. On the other hand, at 55d, $S+F_{3(45)}$ gave minimum enzyme activity which differed critically from those for the remaining treatments, except $S+F_{3(35)}$.

4.6.4 Leaf NPK content

Leaf NPK content was determined at 45 and 55d. The combined soaking and spraying of pyridoxine significantly influenced NPK content in leaves. The data are summarised in Table 41 and described below.

4.6.4.1 Nitrogen

Significantly optimum content of nitrogen in leaves (Table 41) was recorded in $S+F_{W(35)}$ and $S+F_{W(45)}$. At 45d, treatment $S+F_{W(35)}$ enhanced the nitrogen content by 1.93% and $S+F_{W(45)}$, by 1.40% and at 55d, $S+F_{W(35)}$ enhanced it by 2.26% and $S+F_{W(45)}$, by 1.82% in comparison with $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively. The minimum value at 45d was obtained in $S+F_{3(35)}$ which significantly differed from those for all other treatments. This treatment gave lowest nitrogen content at 55d also; but the effect was at par with those of $S_W+F_{W(35)}$, $S_W+F_{W(45)}$ and $S+F_{3(45)}$.

Table 41. Combined effect of pyridoxine soaking and spray on leaf NPK content of summer moong var. K-851 (Mean of three replicates).

Treatments	Nitrogen (%)		Phosphorus (%)		Potassium (%)	
	Days after sowing					
	45	55	45	55	45	55
$S_W+F_{W(35)}$	3.930	2.340	0.294	0.235	2.087	1.875
$S+F_{W(35)}$	5.860	4.600	0.368	0.276	2.301	2.163
$S+F_{1(35)}$	3.550	3.320	0.258	0.207	2.115	1.940
$S+F_{2(35)}$	3.380	3.070	0.211	0.196	1.800	1.571
$S+F_{3(35)}$	2.670	2.100	0.183	0.168	1.761	1.562
$S_{W+}F_{W(45)}$	4.170	2.510	0.291	0.229	2.004	1.836
$S+F_{W(45)}$	5.570	4.330	0.384	0.281	2.308	2.200
$S+F_{1(45)}$	-	3.100	-	0.211	-	1.775
$S+F_{2(45)}$	-	3.160	-	0.205	-	1.786
$S+F_{3(45)}$	-	2.430	-	0.179	-	1.643
C.D. at 5%	0.526	0.505	0.027	0.027	0.097	0.150

N.B. Seeds were soaked for 4h and then treated with rhizobium inoculum

Plants were sprayed either at 35 or 45d

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

4.6.4.2 Phosphorus

Significantly maximum phosphorus content also was found in $S+F_{W(35)}$ and $S+F_{W(45)}$ and the values given by these two treatments were at par with each other (Table 41). The increase due to these treatments was 0.07% and 0.09% at 45d and 0.04% and 0.05% at 55d compared with $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively. Lowest phosphorus content in leaves at both stages was recorded in $S+F_{3(35)}$. However, the value at 55d was at par with that for $S+F_{3(45)}$.

4.6.4.3 Potassium

For leaf potassium content (Table 41), $S+F_{W(35)}$ and $S+F_{W(45)}$, showing equal effect, proved significantly optimum at both stages. Treatment $S+F_{W(35)}$ and $S+F_{W(45)}$ enhanced leaf potassium by 0.21% and 0.30% at 45d and by 0.29% and 0.36% at 55d than treatments $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively. At both stages, $S+F_{3(35)}$ exhibited lowest potassium content in leaves. However, its effect was at par with that of $S+F_{2(35)}$ at both stages and with that of $S+F_{3(45)}$ only at 55d.

4.6.5 Yield characteristics

Five yield characters, namely pod number/plant, pod length, seed number/pod, 1,000 seed weight and seed yield, were studied at harvest. All these parameters were significantly

affected by pyridoxine treatment (Table 42). A brief description of the effect on each characteristic is given below.

4.6.5.1 Pod number/plant

Significantly maximum pods were observed in $S+F_{W(35)}$ and $S+F_{W(45)}$, the two treatments showing equal effect. The increase due to $S+F_{W(35)}$ and $S+F_{W(45)}$ was 51.51% and 51.26% over $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively. Treatment $S+F_{3(45)}$ produced significantly lowest number of pods (Table 42).

4.6.5.2 Pod length

$S+F_{W(35)}$ and $S+F_{W(45)}$, having equal effect, proved significantly optimum for pod length (Table 42). These treatments gave 7.30% and 8.20% higher values than $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively. On the other hand, $S+F_{3(45)}$ exhibited significantly shortest pods.

4.6.5.3 Seed number/pod

Among various treatments, $S+F_{W(35)}$ and $S+F_{W(45)}$, giving statistically equal values, produced significantly maximum number of seeds (Table 42). Treatments $S+F_{W(35)}$ and $S+F_{W(45)}$ enhanced seed number by 6.07% and 6.59% in comparison with $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively. Treatment $S+F_{3(35)}$ exhibited lowest number of seeds but its effect was at par with that of $S+F_{3(45)}$.

Table 42. Combined effect of pyridoxine soaking and spray on yield parameters and seed protein content of summer moong var. K-851 (Mean of three replicates).

Treatments	Pod number/ plant	Pod length (cm)	Seed number/ pod	1,000 seed weight (g)	Seed yield (q/ha)	Protein content (%)
$S_W+F_{W(35)}$	9.334	7.207	9.077	43.533	9.430	23.520
$S+F_{W(35)}$	14.142	7.733	9.628	45.400	14.463	24.300
$S+F_{1(35)}$	11.500	7.485	9.133	44.570	11.734	22.560
$S+F_{2(35)}$	10.560	7.547	8.933	47.610	10.595	21.600
$S+F_{3(35)}$	9.900	7.283	8.231	43.250	9.144	19.200
$S_W+F_{W(45)}$	9.256	7.136	9.071	43.377	9.644	23.360
$S+F_{W(45)}$	14.001	7.721	9.669	45.550	14.274	24.000
$S+F_{1(45)}$	12.000	7.453	9.312	44.760	10.016	24.000
$S+F_{2(45)}$	9.115	7.273	8.533	45.620	9.716	21.600
$S+F_{3(45)}$	8.485	6.560	8.267	44.580	9.030	20.100
C.D. at 5%	0.183	0.147	0.169	1.491	0.263	0.495

N.B. Seeds were soaked for 4h and then treated with rhizobium inoculum

Plants were sprayed either at 35 or 45d

A uniform basal dose of 10kg N, 30kg P and 35kg K/ha was applied

4.6.5.4 1,000 seed weight

Maximum 1,000 seed weight was recorded in $S+F_{2(35)}$ and the value differed critically from those for the rest of the treatments (Table 42). This treatment enhanced seed weight by 9.36% compared with $S_W+F_{W(35)}$. On the other hand, $S+F_{3(35)}$ produced lightest seeds. However, its effect was at par with those of $S_W+F_{W(35)}$, $S_W+F_{W(45)}$ and $S+F_{3(45)}$.

4.6.5.5 Seed yield

$S+F_{W(35)}$ and $S+F_{W(45)}$, exhibiting equal effect, resulted in significantly optimum seed yield (Table 42). These treatments increased seed yield by 53.37% and 48.01% compared with $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively. The lowest value was noted in $S+F_{3(45)}$. However, this value was at par with that for $S+F_{3(35)}$.

4.6.6 Seed protein

Significantly maximum seed protein content (Table 42) was obtained in $S+F_{W(35)}$, $S+F_{W(45)}$ and $S+F_{1(45)}$ which were at par in their effect. On the other hand, the values recorded for the latter two treatments were at par with that for $S_W+F_{W(35)}$; but differed critically from that for $S_W+F_{W(45)}$. The increase in protein content due to $S+F_{W(35)}$ and $S+F_{W(45)}$ was 0.78% and 0.64% over $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ respectively. Treatment $S+F_{3(35)}$ gave significantly lowest seed protein content.

4.7 General remarks

During the entire investigation, except in Experiment 2 where the sampling was done only once, the pattern of different characteristics exhibited certain trends as the plants matured. The general trends are described below.

4.7.1 Lentil

1. Plant length, fresh weight and dry weight increased continuously with the advance in age of the plants.
2. In Experiments 1 and 3, root length at the first two samplings remained almost similar but decreased at the third sampling.
3. The root nodule number increased till 90d in Experiments 1 and 3. Thereafter, a decrease in root nodule number was noted in these experiments.
4. In all experiments, leaf number showed an increasing trend till the last sampling.
5. Unlike leaf number, NAR in both the experiments was decreased at the last interval.
6. NRA levels in leaves decreased till the second sampling but showed a slight increase at the last sampling.
7. Leaf NPK content decreased as plants progressed towards maturity.

4.7.2 Summer moong

1. Plant length, fresh weight and dry weight in all experiments on summer moong increased till the last sampling.

2. In Experiment 4, root length increased upto 30d and, thereafter, decreased continuously till the last sampling. However, in Experiments 5 and 6, this parameter increased till the last sampling.

3. In Experiment 4, root nodules were not conspicuous at 20d; but increased till 40d and decreased at the last sampling. On the other hand, root nodules could be observed only at 45d in Experiments 5 and 6; but degenerated at the last sampling.

4. In all experiments, except Experiment 4, leaf number showed an increasing trend till the last sampling. However, in case of Experiment 4, leaf number increased till the penultimate sampling(40d)only.

5. In Experiment 4, the increase in NAR was noted till 30-40d period. On the other hand, in Experiments 5 and 6, this parameter decreased with the successive interval.

6. In Experiment 4, no definite trend in leaf NRA level was noted with the advancing age of plants; but in Experiments 5 and 6, the enzyme activity decreased with the increasing age of plants.

7. Leaf NPK content decreased in all experiments as plants progressed towards maturity.

C H A P T E R - 5

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DISCUSSION

5.1 Introduction

The seed is a resting structure. It is usually extremely dehydrated, composed of storage tissue and necessary factors, including growth regulators. Metabolic processes are arrested or take place very slowly. Thus, the seed is in a state of suspended animation, mainly due to lack of water and oxygen. The process of germination includes absorption of water, reactivation of metabolism and initiation of growth (Bidwell, 1979). In a suitable environment, the seed becomes active and is transformed into a plant. During this transition, balanced supply of different growth regulators, in addition to reserve food materials, plays a crucial role, and a disturbance in this balance may stop germination totally or produce a weak plant. After germination, successful establishment of the seedling becomes important. Luxuriant growth of the early root system helps explore the soil to ensure ample supply of water and nutrients for maintaining normal growth and development of the plant.

The growth of the root system is regulated by certain growth regulators. Briefly, growth regulators play a pivotal role in the beginning of plant life. Of these, pyridoxine has been conclusively proved to enhance germination of various seeds (Noggle and Wynd, 1943; Ovcharov and Kulieva, 1968; Kozin and

Kravtsov, 1973) and to promote the growth of excised roots of various species (Chapter 2). The seed usually contains adequate amount of this vitamin to sustain proper growth of the juvenile root. However, if seeds possess insufficient amount of pyridoxine, the plants arising from them have a poorly developed root system. This results in reduced vegetative and reproductive growth of the entire plant. Subsequently, the economic produce of such plants is also adversely affected. It may, therefore, be argued that if seed treatment with pyridoxine prior to sowing could accelerate its germination and root growth this could finally boost the productivity of the plants. This has already been established at Aligarh for some cereals (Ahmad, 1975; Afridi et al., 1979; Ahmad et al., 1981, 1982; Ashfaq et al., 1983).

However, leguminous crops were not included in this programme, inspite of their indispensability in the daily Indian diet. Preliminary investigations by the author in petridishes revealed that pyridoxine enhanced germination and root growth of lentil and summer moong. The review of literature (Chapter 2) clearly established that this vitamin is pleiotropic in its action, that is, it has more than one effect on the growth and development of plants. Therefore, the effect of pyridoxine application was investigated on growth characteristics, net assimilation rate, nitrate reductase activity, leaf NPK content, yield characteristics and seed protein content of lentil and summer moong. The experiments described in this thesis may be categorised on the following lines.

1. Effect of pre-sowing seed treatment with graded aqueous pyridoxine solution.

2. Effect of foliar spray of graded aqueous pyridoxine solution applied at flower-initiation (90d in lentil and 35d in summer moong) or at fruit-initiation (110d in lentil and 45d in summer moong).

3. Effect of combinations of seed soaking and foliar spray of pyridoxine solution.

5.2 Effect of pre-sowing seed treatment with pyridoxine

Experiments 1 and 4 were conducted to observe the effect of pre-sowing seed treatment with pyridoxine on the performance of lentil var. T-36 and summer moong var. K-851 respectively. The treatments that were common in both trials, consisted of S_W (water soaked), S_1 (0.1%), S_2 (0.2%), S_3 (0.3%), S_4 (0.4%) and S_5 (0.5%). An unsoaked control (S_0) was included in the lentil experiment; but as S_0 and S_W proved at par in their effect on all the parameters studied, it was omitted in that on summer moong. The lentil and summer moong seeds were soaked for 12h and 4h respectively as the latter possessed delicate seed coat which allowed easy entry of the vitamin compared with that of the former. These periods of soaking were determined by preliminary investigations conducted in petridishes. The data of these two experiments are summarised in Tables 9-13 and 25-30 and are briefly discussed below.

5.2.1 Growth characteristics

In Experiment 1 on lentil (Tables 9-10), all growth parameters (plant length, root length, root nodule number, leaf number, fresh weight and dry weight) were significantly affected by pyridoxine treatment, except root length at 90 and 120d. In general, soaking in 0.3% pyridoxine solution (S_3) proved optimum for almost all parameters at all three samplings. However, plant length was maximum in S_1 at three samplings but S_3 followed closely behind it. Moreover, root length, leaf number, fresh weight and dry weight at 60d were also optimum in S_1. In Experiment 4 on summer moong (Tables 25-27), the same growth parameters at all four stages were significantly affected by soaking treatment with pyridoxine, except leaf number, fresh weight and dry weight at 20d. Treatment S_3 proved superior for almost all characteristics. However, plant length and root length at 20d were found maximum in S_1 and S_2 respectively.

It indicates that lentil and summer moong showed similar response to pyridoxine treatment. A simple explanation may be that seeds of these crops contained approximately the same native pyridoxine content, the values being 23.51 µg/g and 22.78 µg/g dry weight of seeds respectively which were seemingly inadequate to sustain normal development of either crop. Therefore, pyridoxine content in seeds may be taken as a criterion to decide whether plants will respond to the vitamin treatment or not. Such studies

have not been conducted so far. However, Bonner and Greene (1939) suggested that leaf vitamin index might be taken as a tool for this purpose.

The data of the present study also point out that the plants, at early stage, required low amount of pyridoxine and various organs needed different amount of this vitamin for their optimum development. Thus, plant length (at all stages), root length, leaf number, fresh weight and dry weight at 60d in Experiment 1 and plant length and root length at 20d in Experiment 4 responded maximally to low concentration of pyridoxine. The differential response at various stages to pyridoxine concentrations supports the idea that plants need specific amount of pyridoxine which varies from organ to organ and stage to stage. In this context, the findings of Thimann (1937), with regard to auxin, may be cited which showed that roots, buds and stems require different amount of auxin for their growth. It appears that such studies have not been conducted with pyridoxine. However, a beneficial effect of this vitamin has been reported on several crops, including legumes, grown in sand culture (Murneek, 1941; Brusca and Haas, 1957; Barbieri, 1959; Zavenyagina and Bukin, 1969; Afridi et al., 1979; Khan and Ansari, 1984). Infact, this vitamin has long been known to be responsible for root growth in vitro (Chapter 2).

Pre-sowing seed treatment with pyridoxine in the present study increased the endogenous level of this vitamin to such an extent that in addition to the successful establishment of the seedling, root growth was sustained for a prolonged period (Tables 9 and 25). In this respect, it is relevant to mention that Fries (1955a) noted adequate amount of vitamins in roots excised from decotylised pea seedlings, indicating that such plants diverted their "hypocotyl vitamins reserve" to the developing roots. The higher fresh and dry weight of plants receiving treatments S_3 (in both experiments) seems to be a manifestation of the beneficial effect of the vitamin at this concentration on root length, root nodule number and leaf number which brought about accelerated uptake of nutrients (Tables 12 and 29). These were then successfully assimilated with the aid of the enhanced photosynthetic activity of leaves (see 5.2.2), thus accounting for the increase in fresh and dry matter production. It may be noted that the highest pyridoxine dose, i.e. S_5 proved supra optimal for most of these growth parameters and this effect was reflected in the other parameters noted below. Lastly, the decrease in root length and root nodule number in both experiments and in leaf number in Experiment 4 might be due to senescence leading to degeneration of these organs.

5.2.2 Net assimilation rate (NAR)

As some nine-tenth of the dry weight of a plant arises directly from photosynthesis, it is logical to explore the effects of the applied vitamin on growth rates in terms of the size of the photosynthesising surface (taken as the area of green leaves) and the intensity or efficiency at which each unit of leaf functions. This efficiency is usually determined by computing NAR values which reflect a direct relationship with the yielding ability of a crop. Therefore, NAR was calculated for 60-90 and 90-120d periods in Experiment 1 on lentil (Table 11) and 20-30, 30-40 and 40-50d periods in Experiment 4 on summer moong (Table 28). In both the experiments, NAR was enhanced most by treatment S_3 at each interval. It might be a manifestation of the beneficial effect of this treatment on most of the growth parameters particularly leaf number and dry weight in both crops. In the experiment on lentil, a decrease in NAR was noted at the second time interval. It was presumably due to an increase in leaf number with the growth of the plants (Table 10) which might have resulted in mutual shading of leaves to such an extent that it prevented maximum harvesting of solar radiation with consequent decrease in the amount of photosynthates formed (Milthorpe and Moorby, 1979). However, in summer moong, the leaves were fewer in comparison with lentil which did not make the shading so effective. Therefore, NAR continued to increase parallel to leaf number upto 30-40d interval. The decrease in NAR noted at 40-50d in this crop may be the result of abscission of

leaves and senescence as the plants reached maturity (Table 26). Treatment T_5, being supra-optimal for most of the growth characters, decreased NAR also in both the crops, as would be expected.

5.2.3 Nitrate reductase activity (NRA)

Plants absorb large amount of inorganic nitrogen from the soil in the form of nitrates. These are reduced to ammonia before being incorporated via simple amino acids into the more complex organic molecules like proteins and nucleic acids. The first step in this sequence of reactions is regulated by nitrate reductase (E.C. 1.6.6.1) which reduces nitrate to nitrite. The leguminous crops are also endowed with another mechanism, i.e. dinitrogen fixation through symbiotic nitrogenase system. However, being more rapid and convenient, the assay of NRA was preferred over that of nitrogenase. Treatment S_3 gave highest NRA in leaves of lentil and summer moong at all stages of sampling (Tables 11 and 28). Such studies, regarding the effect of pyridoxine alone, on NRA are lacking in the literature. However, combined treatment of thiamine and pyridoxine has been found to avert zinc deficiency and to reduce nitrate accumulation in zinc deficient tomato plants; but NRA remained unaltered in these experiments (Davydova, 1966). In the present investigations, the plausible explanation seems to be that pyridoxine accelerated uptake of nitrate by the roots of lentil and summer moong by

providing larger surface area for absorption (Tables 12 and 29). It might have resulted in higher quantity of nitrate reductase which not only requires its substrate (nitrate) for the induction of the enzyme but also for its stability (Hewitt and Afridi, 1959; Afridi and Hewitt, 1962). The alternative possibility may be that the vitamin directly induces the synthesis of nitrate reductase. Such direct induction of NRA by GA or GA + cytokinin in tobacco leaves and by cytokinin in embryos of Agrostemma githago and in cucumber cotyledons has been observed by several workers (Roth-Bejerano and Lips, 1970; Kende et al., 1971; Hirschlery et al., 1972; Kende and Shen, 1972; Knypl, 1973). The highest dose of pyridoxine in Experiment 4 on summer moong proved inhibitory for NRA which indicates that lentil NRA (Experiment 1) may tolerate comparatively higher doses of pyridoxine.

5.2.4 Leaf NPK content

In both the experiments, leaf NPK content at all stages was significantly affected by pyridoxine treatments, except that of potassium in summer moong at 50d (Tables 12 and 29). Among various treatments, S_3 proved optimum for all three nutrients. The role of B-vitamins in promoting nutrient uptake by plants is comparatively little explored. However, Ovcharov and Kulieva (1968) reported higher content of nitrogen and phosphorus in 48h old cotton seedlings as a result of soaking of seeds in pyridoxine. Similarly, enhanced uptake of nitrogen and phosphorus in

Mentha piperita and of several elements, including NPK, in Vigna radiata seedlings was observed as a result of treatment with B-vitamins, including thiamine, pyridoxine and nicotinic acid (Dimitrova-Russeva and Lilova, 1969; Gopala Rao and Raghava Reddy, 1985). It seems likely that pyridoxine treatment might have facilitated the entry of these nutrients by increasing the permeability of the root cell membrane. Alternatively, pyridoxine might have behaved like the co-enzyme of certain carrier proteins which are considered to be responsible for transporting the nutrients across the membrane. At our present state of knowledge, these assumptions may not be verified. However, it may be recalled that the role of pyridoxine as co-enzyme in aminotransferases and transaminases is well established (Lehninger, 1982).

Considering NPK content of leaves at succeeding growth stages in both the crops, a decrease in concentration of all these nutrients was noted with the increase in the age of the crop. Ordinarily, this situation arises as a result of "dilution with growth" due to an exponential increase in growth (weight and volume) of plants. Consequently, high quantities of nutrients appear to be less when expressed on per unit basis (Lundegårdh, 1951). Moreover, the decrease in leaf NPK concentration at later stages may be due to the translocation of these nutrients from the vegetative parts (source) to the developing pods (sink). A similar step-wise decline in the amount of the nutrients at later growth stages has also been reported in leguminous crops by Singh

and Singh (1983), Arora and Luthra (1971), Venugopal and Morachan (1974) and Paricha et al. (1983).

5.2.5 Yield characteristics

All the yield parameters, except pod length in Experiment 1, were significantly enhanced by pyridoxine treatment. A similar beneficial effect of pyridoxine on yield parameters of barley and triticale was noted by Afridi et al. (1979) and Ashfaq et al. (1983) respectively. In the present study, treatment S_3 proved optimum for these parameters, except 1,000 seed weight, which was decreased in both experiments. The decrease in test weight seems to be due to dilution effect as the treatment resulted in the differentiation of more pods/plant and seeds/pod with the consequent distribution of the metabolites to a larger number of sinks (seeds). This is supported by the ratio of dry weight, particularly at the last stage to (pods x seeds/pod) for the control (S_W) and optimum treatment (S_3) in both the experiments, the corresponding values for S_W and S_3 being 0.050 and 0.047 at 120d in lentil and 0.058 and 0.042 at 50d in summer moong.

In these two experiments, seed yield also was found highest in treatment S_3. Such beneficial effect of B-vitamins, including pyridoxine, on productivity of several crops has been reported by other workers also (Kjelvik, 1965; Aĭzikovick, 1967; Filimonov, 1967; Sinkovics, 1970; Mikhailova, 1974; Polyanskaya

and Kuvadov, 1974; Sinkovics, 1974; Ahmad et al., 1982; Ashfaq et al., 1983). As mentioned earlier, treatment S_3 also proved optimum for most of the growth characteristics, NAR, NRA, leaf NPK content and most of the yield characteristics. It clearly indicates that the cumulative effect of these parameters resulted in enhanced seed yield in the two crops. This view is further strengthened by the correlation studies of these parameters against seed yield in both the experiments (Tables 43 and 46). These reveal that correlation of growth parameters, with the exception of root nodules, with yield did not follow any definite trend; but NAR and NRA at all samplings showed strongest correlation ($p < 0.01$) with seed yield. Information regarding correlation between growth parameters and seed yield in lentil and summer moong is scarce (Akhtar, 1985). Correlation of NAR with seed yield has been established for the first time in the present studies. However, NRA is known to be significantly correlated with seed yield of summer moong (Akhtar et al., 1984). Some other workers have also reported similar correlations between NRA and seed yield in cereals (Deckard et al., 1973; Johnson et al., 1976; Dalling and Loyan, 1977). Like NRA, leaf NPK content also exhibited correlation with seed yield; but the trend differed slightly in the two crops. In lentil, though leaf NPK at all stages was correlated with seed yield, the order of correlation was potassium > nitrogen > phosphorus. In summer moong on the other hand, phosphorus was correlated with seed yield at all stages while nitrogen and potassium did not show

Table 43. Correlation of various parameters with seed yield and seed protein content of lentil var. T-36.

(number of observations 21)

Parameters	Days	Correlation coefficient (r)	
		Yield	Protein
Growth parameters			
Plant length	60	0.515*	
	90	N.S.	
	120	0.527*	
Root length	60	0.493*	
	90	N.S.	
	120	N.S.	
Nodule number	60	0.549*	
	90	0.524*	
	120	0.894**	
Leaf number	60	N.S.	
	90	0.706**	
	120	0.719**	
Fresh weight	60	0.443*	
	90	0.689**	
	120	0.600**	
Dry weight	60	0.660**	
	90	0.831**	
	120	0.637**	
Net assimilation rate	60-90	0.772**	
	90-120	0.570**	
Nitrate reductase activity	60	0.839**	0.867**
	90	0.738**	0.840**
	120	0.810**	0.958**
Leaf NPK content			
Nitrogen	60	0.941**	0.839**
	90	0.876**	0.726**
	120	0.446*	N.S.
Phosphorus	60	0.754**	0.886**
	90	0.454*	0.928**
	120	0.812**	0.932**
Potassium	60	0.894**	0.802**
	90	0.929**	0.862**
	120	0.979**	0.876**
Yield parameters			
Pod	140	0.791**	
Pod length	140	N.S.	
Seed number	140	0.597**	
1,000 seed weight	140	N.S.	

* Significant at 5%; ** Significant at 1%; N.S. Non-significant.

Table 46. Correlation of various parameters with seed yield and seed protein content of summer moong var. K-851.

(number of observations18)

Parameters	Days	Correlation coefficient (r) Yield	Protein
Growth parameters			
Plant length	20	N.S.	
	30	0.796**	
	40	N.S.	
	50	0.536*	
Root length	20	N.S.	
	30	0.817**	
	40	0.550*	
	50	N.S.	
Nodule number	20	No root nodules	
	30	0.902**	
	40	0.676**	
	50	N.S.	
Leaf number	20	N.S.	
	30	0.558*	
	40	0.510*	
	50	0.574*	
Fresh weight	20	N.S.	
	30	0.643**	
	40	0.533*	
	50	N.S.	
Dry weight	20	N.S.	
	30	0.661**	
	40	0.546*	
	50	0.568*	
Net assimilation rate	20-30	0.742**	
	30-40	0.774**	
	40-50	0.792**	
Nitrate reductase activity	20	0.805**	N.S.
	30	0.527*	0.713**
	40	0.577*	0.935**
	50	0.770**	0.717**
Leaf NPK content			
Nitrogen	20	0.525*	0.544*
	30	0.469*	0.556*
	40	0.576*	0.590**
	50	N.S.	0.483*
Phosphorus	20	0.617**	0.712**
	30	0.604**	0.704**
	40	0.656**	0.740**
	50	0.565*	0.633**
Potassium	20	0.848**	0.861**
	30	0.881**	0.855**
	40	0.888**	0.926**
	50	N.S.	N.S.
Yield parameters			
Pod number	62	0.616**	
Pod length	62	0.738**	
Seed number	62	0.755**	
1,000 seed weight	62	N.S.	

*Significant at 5%; **Significant at 1%; N.S. Non-significant.

any correlation at 50d. The order of correlation also was changed: phosphorus > potassium > nitrogen. Therefore, potassium and phosphorus in leaves at early stages may be utilised for predicting seed yield in lentil and summer moong respectively. Correlation of leaf NPK content with seed yield has been established in these crops by Akhtar (1985) also. Further, it was noted that pod number and seeds/pod in lentil and pod number, pod length and seeds/pod in summer moong showed strongest correlation ($p < 0.01$) with seed yield. The contribution of pod number and seeds/pod to seed yield is obvious and well established. Moreover, correlation of pod length with seed yield in summer moong appears to indicate that photosynthesis in the pod wall might have helped in filling the grains possibly by utilising the additional nutrients translocated from the leaves (p.139). Similar observations have been reported in chickpea and soybean by Thorne (1978, 1979) and Bangal et al. (1983). Moreover, strong association between yield parameters and seed yield has also been established in different other legumes by several workers (Singh and Singh, 1969; Beohar and Nigam, 1972; Tomar et al., 1973; Dixit and Singh, 1975; Bhaumik and Jha, 1976; Singh and Singh, 1976; Saraswathy et al., 1979; Tikka and Asawa, 1981; Chauhan and Sinha, 1982; Sarwar et al., 1982).

5.2.6 Protein content

In Experiments 1 and 4, the highest protein content in seeds was recorded in treatments S_3 and S_2 respectively. It may be recalled here that optimum seed yield in both the experiments was obtained in S_3; but the magnitude of this increase in yield was higher in Experiment 4 (Table 30) than in Experiment 1 (Table 13). It seems that increased yield due to this treatment in Experiment 4 might have caused dilution of protein in these seeds and, hence, S_2 surpassed S_3 for protein content of summer moong. This would become evident by comparing the computed value of total seed protein in S_2 and S_3, the values being 2.681 and 3.465 q/ha respectively.

The beneficial effect of pyridoxine on protein content is not altogether surprising as this vitamin, being a co-enzyme of aminotransferases and transaminases, helps the synthesis of amino acids by utilising the organic acids produced during oxidation of carbohydrates in Krebs Cycle (Lehninger, 1982). The amino acids so produced are incorporated into the protein. Such a shift in metabolic pathways appears when there is ample supply of nitrogen accompanied by greater availability of phosphorus in the form of the energy-rich ATP (Hewitt, 1963) and of potassium for peptide synthesis (Webster, 1956). In the present study also, pyridoxine was found to increase the uptake of these nutrients in lentil and summer moong (Tables 12 and 29). Further, the observed

correlations of NRA and leaf NPK content with seed protein content in both the experiments strengthen this argument. Studies concerning pyridoxine and improvement in seed quality of legumes have not been carried out so far. However, Ahmad (1975) and Afridi *et al.* (1979) noted similar positive effect of pyridoxine on seed protein content of several barley cultivars in sand culture as well as under field conditions.

5.3 Effect of pyridoxine spray

Experiments 2 and 5 were conducted side by side with Experiments 1 and 4 on lentil and summer moong respectively. The spray of pyridoxine solution was done on leaves either at flower-initiation or fruit-initiation stages in these crops. However, in Experiment 5, unsprayed control was excluded as it had given values equal to those for water-sprayed control in Experiment 2. The data of the two experiments are summarised in Tables 14-18 and 31-36 respectively. The results on growth parameters, NAR, NRA, leaf NPK content, yield parameters and seed protein content are discussed below.

5.3.1 Growth characteristics

In Experiment 2 (Tables 14-15), all the growth parameters were significantly affected by spray treatments. Of these, $F_{2(90)}$ proved optimum for the growth parameters in lentil. Similarly, in Experiment 5 (Tables 31-33), all the growth characteristics, except

root length at 55d were significantly affected by pyridoxine spray. Among various treatments, $F_{3(35)}$ proved optimum for growth performance of summer moong. The results indicate that lentil requires higher dose of pyridoxine spray than summer moong. It may be due to the pinnately compound nature of the leaves of lentil with very small leaflets which may allow a part of the sprayed pyridoxine solution to percolate down without being fully absorbed. It may be pointed out that, although soaking of lentil seeds in pyridoxine solution (Experiment 1) showed no effect on root growth at 120d (Table 9), spray of the vitamin (Experiment 2) significantly enhanced it (Table 14). This observation is contrary to that on summer moong, in which seed soaking had significant effect on root growth at 50d (Table 25). It seems likely that, lentil being a long duration crop, the effect of seed soaking on root growth could not be sustained till 120d, whereas in summer moong that was harvested much earlier (62d), this effect could persist. The significant positive effect of spray in both crops (Tables 14 and 31), particularly in lentil, is, therefore, understandable as pyridoxine supplied by foliar spray at late growth stages was able to sustain root growth.

Let us now consider the response of other vegetative characters of lentil and summer moong to pyridoxine sprayed at 90 and 35d (flower-initiation stage) respectively (Tables 14-15 and 31-33). The observed data suggest that a competition between vegetative and reproductive organs for utilising the available

pyridoxine in leaves started at this stage and it is probable that the plants preferred to divert their vitamin to the developing reproductive parts. In this situation, the roots, being remotely situated, presumably did not get adequate supply of the vitamin and exhibited poor development. Spray of pyridoxine at this stage would be expected to increase the total quantity of the vitamin in the leaves and thus ensure better supply to the roots to sustain their growth, like that of the other vegetative parameters. On the other hand, it is fairly established that vegetative growth ceases almost completely at fruit-initiation stage (Bidwell, 1979). The application of the vitamin at this stage (110d and 45d in lentil and summer moong respectively) seems, therefore, to promote reproductive growth only, as reflected by pod number, pod length, seeds/pod etc. (Tables 18 and 36).

The effect of pyridoxine spray on leguminous crops has not been observed so far. However, Kŭdrev and Pavlov (1965) observed that pyridoxine spray averted the ill effects of flooding at tillering, shooting and heading stages in wheat. Similarly, Arsen'eva (1977) reported beneficial effect of pyridoxine on shoot growth of lilac.

5.3.2 Net assimilation rate (NAR)

Like growth characteristics, NAR was significantly optimum in treatments $F_{2(90)}$ and $F_{3(35)}$ in lentil and summer moong

respectively (Tables 16 and 34). As discussed earlier (p.136), NAR is the indicator of dry matter accumulation due to unit photosynthetic area in unit time. In the present study (Experiments 2 and 5), accumulation of more dry matter and production of more leaves in treatments $F_{2(90)}$ and $F_{3(35)}$ might have resulted in optimum NAR in lentil and summer moong respectively as the treatment increased these parameters maximally (Tables 15 and 32-33). Such observations are lacking in the literature with regard to pyridoxine application. However, thiamine, another member of B-vitamins, was found to enhance photosynthesis in kidney beans and cabbages (Iijima, 1957b).

5.3.3 Nitrate reductase activity (NRA)

Spray of $F_{2(90)}$ and $F_{3(35)}$ in Experiments 2 and 5 respectively proved optimum for NRA (Tables 16 and 34). The plausible reasons for enhanced NRA levels as a result of pyridoxine treatment have been discussed on p.137. However, such an effect of pyridoxine spray on nitrate reductase activity has not been recorded earlier. The observation of Kŭdrev and Pavlov (1965) that pyridoxine spray corrected the disturbed nitrogen metabolism in flooded wheat, in my opinion, might have been due to enhanced NRA as observed in the present experiments. It may also be added that Sruoginite and Shpokene (1968) and Sruoginite (1980) observed a positive effect of the spray of B-vitamins, particularly riboflavin, on restoring the carbonic anhydrase activity depressed by inhibitors in Phaseolus vulgaris and Avena sativa.

5.3.4 Leaf NPK content

Regarding leaf NPK content (Table 17) in lentil (Experiment 2), maximum leaf nitrogen and phosphorus content were found in $F_{2(90)}$ and $F_{1(110)}$ (being statistically equal), indicating that the uptake of these two nutrients responded more to higher concentration of the vitamin at flower-initiation than at fruit-initiation stage, while potassium content in leaves responded to lower dose of pyridoxine (0.1%) sprayed at either stage. In Experiment 5 (Table 35), however, spray of 0.1% pyridoxine at flower-initiation stage, i.e. $F_{3(35)}$, invariably proved optimum for leaf NPK content. Phosphorus content in leaves was optimum in this treatment applied at fruit-initiation stage also. These findings are expected as flower-initation is a stage of great metabolic activity and is consequently influenced considerably by external stimuli. Infact, at this stage, the meristem of vegetative bud is converted into reproductive bud. The enhanced leaf NPK content might be a manifestation of some hitherto unexplored physiological role of pyridoxine leading to change in permeability of cell membrane of roots or to higher activity of certain enzymes responsible for the uptake of these nutrients as discussed earlier (p.139).

Kudrev and Pavlov (1965) and Kŭdrev and Pandev (1967) observed that pyridoxine spray not only normalised the disturbed nitrogen metabolism but also enhanced nitrogen uptake in flooded

wheat; but a beneficial effect of pyridoxine spray on phosphorus and potassium uptake is not yet reported. However, Almestrand (1951), using excised roots of cereals, noted increased uptake of phosphorus from the medium when pyridoxine was included.

5.3.5 Yield characteristics

In Experiment 2 (Table 18), all yield characteristics, except 1,000 seed weight, were significantly affected by pyridoxine spray. Among different spray treatments, $F_{2(90)}$ and $F_{2(110)}$ proved optimum. On the other hand, in Experiment 5 (Table 36), the beneficial effect of pyridoxine spray on various yield attributes was specific with regard to the dose and stage. For instance, pod number and 1,000 seed weight were optimum in $F_{3(35)}$ and $F_{1(45)}$ respectively. While pod length and seed number/pod were highest in 0.1% pyridoxine sprayed at either of the two stages, i.e. $F_{3(35)}$ and $F_{3(45)}$. The observation that pyridoxine sprayed at fruit-initiation stage was beneficial for all yield attributes in Experiment 2 and for pod length and seeds/pod in Experiment 5, supports the assumption made on p. 146 in relation to partitioning of metabolites for reproductive phase. The variable response of the two crops to the concentration of pyridoxine spray at varying stages may, however, be the manifestation of their genetical make up.

Seed yield in both these experiments corresponded to the effect on growth and yield attributes. Thus, highest seed yield was recorded in $F_{2(90)}$ and $F_{2(110)}$ in lentil and $F_{3(35)}$ in summer moong. These results further verify that yield is the manifestation of the vegetative and reproductive traits and correlation studies (Tables 44 and 47) also support this generalisation. Similar beneficial effect of pyridoxine spray has been observed in a few crops, including wheat, Capsicum annuum and lilac (Kŭdrev and Pavlov, 1965; Popova et al., 1971; Arsen'eva, 1977).

5.3.6 Protein content

In Experiment 2 (Table 18), pyridoxine spray at either stage was equally effective for seed protein content. Among various spray treatments, $F_{2(90)}$, equalled by $F_{1(90)}$ and $F_{1(110)}$ proved optimum. However, in Experiment 5, 0.1% pyridoxine sprayed at flower-initiation stage, i.e. $F_{3(35)}$, gave highest protein content in seeds. This seemed to be the manifestation of increased nutrient supply for the synthesis of protein in seeds as a result of their higher uptake as discussed earlier (pp.143 and 148). Strong correlation ($p < 0.01$) of NRA and leaf NPK content with seed protein (Tables 44 and 47) suggests that pyridoxine spray might have regulated and promoted nitrogen metabolism through these effects.

Table 44. Correlation of various parameters with seed yield and seed protein content of lentil var. T-36. (number of observations 39)

Parameters	Days	Correlation coefficient (r)	
		Yield	Protein
Growth parameters			
Plant length	120	0.818**	
Root length	120	N.S.	
Nodule number	120	0.909**	
Leaf number	120	0.666**	
Fresh weight	120	0.755**	
Dry weight	120	0.711**	
Net assimilation rate	90-120	0.555**	
Nitrate reductase activity	120	N.S.	0.686**
Leaf NPK content			
Nitrogen	120	0.463**	0.893**
Phosphorus	120	0.765**	0.767**
Potassium	120	0.770**	0.839**
Yield parameters			
Pod number	140	0.921**	
Pod length	140	0.363*	
Seed number	140	0.770**	
1,000 seed weight	140	N.S.	

*Significant at 5%; **Significant at 1%; N.S. Non-significant.

Table 47. Correlation of various parameters with seed yield and seed protein content of summer moong var. K-851.

(number of observations at 45d= 18; at 55d= 30)

Parameters	Days	Correlation coefficient (r)	
		Yield	Protein
Growth parameters			
Plant length	45	0.917**	
	55	0.716**	
Root length	45	0.506*	
	55	0.583**	
Nodule number	45	0.537*	
	55	Nodules degenerated	
Leaf number	45	0.870**	
	55	0.794**	
Fresh weight	45	0.928**	
	55	0.540**	
Dry weight	45	0.834**	
	55	0.690**	
Net assimilation rate	35–45	0.821**	
	45–55	0.401*	
Nitrate reductase activity	45	0.623**	0.618**
	55	0.851**	0.660**
Leaf NPK content			
Nitrogen	45	0.453*	0.752**
	55	0.672**	0.804*
Phosphorus	45	0.801**	0.916**
	55	0.698**	0.860**
Potassium	45	0.632**	0.648**
	55	0.749**	0.886**
Yield parameters			
Pod number	62	0.748**	
Pod length	62	0.727**	
Seed number	62	0.598**	
1,000 seed weight	62	N.S.	

*Significant at 5%; **Significant at 1%; N.S. Non-significant.

5.4 Combined effect of seed soaking and spray of pyridoxine

Experiments 3 and 6 were conducted on the basis of the results of Experiments 1 and 2 on lentil and 4 and 5 on summer moong respectively. The object was to investigate the combined effect of seed soaking and spray of pyridoxine on the performance of lentil and summer moong. The time for spray (90d for lentil and 35d or 45d for summer moong) was selected on the basis of the data of Experiments 2 and 5 respectively. The results of these experiments are summarised in Tables 19-24 and 37-42 and are discussed below.

5.4.1 Growth characteristics

In Experiment 3 (Tables 14-21), all the growth parameters at 60d were significantly optimum in S_2+F_W. At 90 and 120d, highest values of all these parameters, except root length at 120d, were recorded in S_3+F_W. Root length was maximally enhanced in S_W+F_3. Further, all soaking treatments of pyridoxine supplemented with pyridoxine spray treatments proved inferior. In Experiment 6 (Tables 37-39), the growth parameters, except height (at 45d), leaf number (at 45 and 55d) and fresh weight (at 55d) were highest in $S+F_{W(35)}$ and $S+F_{W(45)}$. Plant length (at 45d) and fresh weight (at 55d) required spray of 0.1% pyridoxine at 35d to supplement the optimum soaking treatment, i.e. $S+F_{1(35)}$, for optimum performance. Other spray treatments either proved

inhibitory or gave equal values to those in soaking treatment only, e.g. $S+F_{1(35)}$ and $S+F_{2(35)}$ for root length, fresh weight and dry weight at various stages. It may also be noted that soaking of seeds in pyridoxine generally exhibited better effect than that of spray treatments in both crops. This is presumably because of the fact that fully expanded leaves are themselves able to synthesise the vitamin (Bonner and Dorland, 1943a), whereas seeds of these crops seem to have less than sufficient quantity (p.133). This is also borne out by the observation that although there was good response to pyridoxine spray at low concentrations, higher doses proved supra-optimal.

As discussed earlier (p.133), it seems that the quantity of the vitamin in the seeds of these crops is not adequate: hence the observed positive response of vegetative parts to exogenous supply of pyridoxine through soaking. The present data also provide strong indirect evidence that flower-initiation stage is another critical period when pyridoxine availability becomes limited to the vegetative parts, especially roots situated remotely from the site of the vitamin synthesis, i.e. leaves, as discussed on pp.145-146. The spectacular response (Table 19) of root length to foliar spray of the vitamin, particularly on lentil, at this stage (compare S_W+F_3 with S_W+F_W) is, therefore, understandable. Further, the observation in summer moong that leaf production (Table 38) responds better when seed soaking is supplemented by pyridoxine spray in contrast to the data on other parameters, e.g.

root length (Table 37), confirms the inference drawn earlier that various organs require different amounts of the vitamin for their optimum development (p.134).

5.4.2 Net assimilation rate (NAR)

Like growth parameters, NAR was optimum in 0.3% pyridoxine soaking plus water spray at 90d, i.e. S_3+F_W during both intervals in Experiment 3 (Table 22). The supplemental spray treatments of pyridoxine proved deleterious. Similarly, in Experiment 6 (Table 40), optimum NAR at both intervals was recorded in 0.3% pyridoxine soaking plus water spray either at 35d or 45d, i.e. $S+F_{W(35)}$ and $S+F_{W(45)}$ respectively. The enhanced NAR due to 0.3% pyridoxine soaking in these experiments, seemed to be the expression of better growth of the plants in the same treatment as discussed earlier (p.136). These findings also confirm the results of Experiments 1 and 4.

5.4.3 Nitrate reductase activity (NRA)

NRA levels in both Experiment 3 and 6 were optimum in 0.3% soaking plus spray of water, i.e. S_3+F_W in Experiment 3 (Table 22) and $S+F_{W(35)}$ and $S+F_{W(45)}$ in Experiment 6 (Table 40). These findings confirm the results of Experiments 1 and 4. The plausible explanation regarding the positive effect of pyridoxine on NRA has been discussed on pp.137-138.

5.4.4 Leaf NPK content

In Experiment 3 (Table 23), leaf NPK content at all stages was optimum in S_3+F_W. However, spray of pyridoxine with soaking proved supra-optimal or gave equal effect to that of pyridoxine soaking. Similarly in Experiment 6 (Table 41), $S+F_{W(35)}$ and $S+F_{W(45)}$ resulted in highest leaf NPK content at both stages. This again testifies that soaking of seeds with pyridoxine is better than combined treatment of pyridoxine soaking and spray, which proved inhibitory.

5.4.5 Yield characteristics

In Experiment 3 (Table 24), soaking in 0.3% pyridoxine solution plus water spray at 90d, i.e. S_3+F_W, invariably enhanced all yield attributes, except 1,000 seed weight. However, water soaking or sub-optimal pyridoxine soaking + spray of pyridoxine, e.g. S_W+F_2 and S_2+F_3 for pod length and S_W+F_2 and S_2+F_1 for seeds/pod proved at par with S_3+F_W. It confirms the earlier mentioned view (p.146) that the pyridoxine supply by spray at flower-initiation favours reproductive growth. In Experiment 6 (Table 42), soaking in 0.3% pyridoxine solution plus water spray at 35d or 45d, i.e. $S+F_{W(35)}$ and $S+F_{W(45)}$, gave highest values for yield parameters, except 1,000 seed weight which was optimum in $S+F_{2(35)}$. It indicates that during filling of the grains, extra amount of pyridoxine is required and confirms the validity of the aforesaid argument.

In conformity with the vegetative and reproductive growth performance, seed yield was optimum in 0.3% pyridoxine soaking plus water spray, i.e. S_3+F_W in Experiment 3 and $S+F_{W(35)}$ and $S+F_{W(45)}$ in Experiment 6. This is further confirmed by observed correlations of various parameters with seed yield in both the experiments (Tables 45 and 48).

From a perusal of the entire data of Experiments 3 and 6, it is clear that soaking of seeds in pyridoxine solution is an efficient way to augment the performance of lentil and summer moong. It is also economical as very small amount of pyridoxine is required for soaking the seeds compared with that for spraying. Further, the technique is convenient and can be adjusted with routine practice of inoculating the seeds with rhizobium.

5.4.6 Protein content

In Experiment 3, optimum protein content in lentil seeds was recorded in S_3+F_W (Table 24). Similarly, $S+F_{W(35)}$ and $S+F_{W(45)}$ enhanced seed protein content in Experiment 6 on summer moong (Table 42), possibly due to the enhanced physiological activities of the plants which might have been triggered by this treatment as discussed earlier (p.143). The observed strong correlation ($p < 0.01$) of seed protein content with NRA and leaf NPK content at all stages, except with phosphorus at 120d in lentil and with potassium at 55d in both experiments (Tables 45 and 48), adds weight to this argument.

Table 45. Correlation of various parameters with seed yield and seed protein content of lentil var. T-36.

(number of observations at 60 and 90d= 12; at 120d= 48)

Parameters	Days	Correlation coefficient (r)	
		Yield	Protein
Growth parameters			
Plant length	60	N.S.	
	90	0.924**	
	120	0.364**	
Root length	60	N.S.	
	90	N.S.	
	120	N.S.	
Root nodule	60	0.909**	
	90	0.956**	
	120	0.397**	
Leaf number	60	N.S.	
	90	0.803**	
	120	N.S.	
Fresh weight	60	N.S.	
	90	0.817**	
	120	0.394**	
Dry weight	60	N.S.	
	90	0.852**	
	120	0.361**	
Net assimilation rate	60-90	0.864**	
	90-120	0.435**	
Nitrate reductase activity	60	0.819**	0.956**
	90	0.900**	0.982**
	120	0.310*	0.656**
Leaf NPK content			
Nitrogen	60	0.861**	0.933**
	90	0.904**	0.579*
	120	N.S.	0.558**
Phosphorus	60	0.964**	0.903**
	90	0.827**	0.887**
	120	0.308*	N.S.
Potassium	60	0.948**	0.773**
	90	0.939**	0.953**
	120	N.S.	0.743**
Yield parameters			
Pod number	140	0.500**	
Pod length	140	0.441**	
Seed number	140	0.347**	
1,000 seed weight	140	N.S.	

*Significant at 5%; **Significant at 1%; N.S. Non-significant.

Table 48. Correlation of various parameters with seed yield and seed protein content of summer moong var. K-851.

(number of observations at 45d= 21; at 55d= 30)

Parameters	Days	Correlation coefficient (r)	
		Yield	Protein
Growth parameters			
Plant length	45	0.726**	
	55	0.769**	
Root length	45	0.765**	
	55	0.762**	
Nodule number	45	0.938**	
	55	Nodules degenerated	
Leaf number	45	N.S.	
	55	0.429*	
Fresh weight	45	0.704**	
	55	0.918**	
Dry weight	45	0.793**	
	55	0.611**	
Net assimilation rate	35–45	0.975**	0.680**
	45–55	0.403*	0.737**
Nitrate reductase activity	45	0.959**	0.680**
	55	0.910**	0.737**
Leaf NPK content			
Nitrogen	45	0.848**	0.851**
	55	0.924**	0.614**
Phosphorus	45	0.777**	0.892**
	55	0.792**	0.844**
Potassium	45	0.807**	0.871**
	55	0.559**	N.S.
Yield parameters			
Pod number	62	0.721**	
Pod length	62	0.751**	
Seed number	62	0.444*	
1,000 seed weight	62	N.S.	

*Significant at 5%; **Significant at 1%; N.S. Non-significant.

5.5 Conclusion

The foregoing discussion clearly indicates a few features regarding the role of pyridoxine in the growth and development of leguminous crops. On the basis of these findings, the following conclusion may be drawn.

1. Pyridoxine content in seeds may be taken as a criterion to predict whether plants will respond to soaking treatments or not. Seedling emergence seems to be the first critical period when exogenous supply of the vitamin becomes imperative to augment the performance of crops with low seed pyridoxine content.

2. Pre-sowing soaking treatment with pyridoxine exhibits beneficial (albeit variable) effect on different organs throughout the entire life of the plant.

3. The action of pyridoxine seems to be pleiotropic with the vitamin influencing various organs independently, though synergism between different organs cannot be ruled out. Further, pyridoxine does not alter the course of growth and development of the plants but enhances these processes.

4. The various organs of the plant require different amount of pyridoxine for their optimum growth and development.

5. Flower-initiation stage seems to be another critical period for pyridoxine application. At this stage, competition starts between vegetative and reproductive growth for utilising the vitamin per se.

6. The growth characteristics, NAR, NRA, leaf NPK content and yield characteristics may be used for predicting the productivity of lentil and summer moong. However, NAR, NRA, leaf NPK content and yield characteristics, showing consistent strong correlation with seed yield, and NRA and leaf NPK content, with seed protein content, are more reliable.

7. The optimum concentration of pyridoxine for seed soaking for most of the parameters, including seed yield and quality in lentil and summer moong, was found to be 0.3%, while the optimum spray treatment was found to be 0.2% and 0.1% (at flower-initiation stage) in lentil and summer moong respectively. The higher concentrations used for soaking or spray appear to be either inhibitory or ineffective. Further, soaking proved superior and economical over spray.

It is, therefore, concluded that pre-sowing seed treatment with 0.3% pyridoxine solution may be exploited commercially to augment the yield and improve the seed quality of lentil and summer moong. Large-scale adoption of the technique by the farmers, therefore, could be expected to overcome the prevailing protein malnutrition in our country to a great extent.

5.6 Proposals for future work

During the course of the present study, it was realised that a number of problems relating to the role of pyridoxine in the physiology of growth and development of plants in general and of legumes in particular, remain to be solved. For example, the presumption of the author that pyridoxine may act, either as hormone enhancing root growth and permeability of cell membrane, or as a co-enzyme of certain enzymes responsible for NPK uptake, may be verified. Similarly, it would be desirable to work out the basic role of pyridoxine in legume-rhizobial symbiosis as the vitamin was found to increase the root nodule number. It would be interesting if the exact mechanism by which pyridoxine influences NRA is investigated at the molecular level as the vitamin could possibly be involved in the NR gene expression. Lastly, from practical point of view, the efficacy of pyridoxine (and other B-vitamins) in improving the yield and quality of various crops of economic importance may be investigated, as encouraging results have been obtained by our group in the present study on legumes and earlier on cereals (Afridi *et al.*, 1979; Ahmad *et al.*, 1981, 1982; Ashfaq *et al.*, 1983).

CHAPTER - 6

SUMMARY

SUMMARY

The importance of the problem "Physiomorphological response of Lens culinaris L. Medic. and Vigna radiata L. Wilczek to pyridoxine application" has been considered briefly. In view of the lacunae in the understanding of the problem, justifications have been put forward for undertaking the present work in Chapter 1.

The available literature on the problem has been reviewed in Chapter 2. The review revealed that vitamins are essential for the growth of excised organs, particularly roots. They enhance the yield of a few cultivated plants when applied through nutrient solution, seed soaking or foliar spray. Among different crops work on legumes, especially lentil and summer moong, and, among vitamins, on pyridoxine is meagre.

The materials and methods used for all the six experiments, performed according to simple randomized block design, have been described with the relevant meteorological and edaphic data in Chapter 3.

The data, mostly found significant at $p < 0.05$ on statistical analysis, have been considered in detail in Chapter 4 and are summarised below.

Experiment 1 (1982-83) was conducted on lentil (Lens culinaris L. Medic.) var. T-36 during "rabi" (winter) season to study the effect of pre-sowing seed treatment for 12h with graded aqueous pyridoxine solution, i.e. 0.0% (S_W), 0.1% (S_1), 0.2% (S_2), 0.3% (S_3), 0.4% (S_4) and 0.5% (S_5) on growth parameters (plant length, root length, root nodule number, leaf number, fresh weight and dry weight), net assimilation rate (NAR), nitrate reductase activity (NRA), leaf NPK content, yield parameters (pod number, pod length, seed number/pod, 1,000 seed weight and seed yield) and seed protein content. An unsoaked control (S_0) was also included in the scheme. The growth parameters, NRA and leaf NPK content were studied at 60, 90 and 120d; NAR was computed for the periods 60-90d and 90-120d and yield parameters and seed protein content were studied at harvest.

Treatment S_3 proved optimum for growth parameters at 90 and 120d; for NAR at both intervals; for NRA and leaf NPK content at all three stages and for yield parameters (except pod length and 1,000 seed weight) and seed protein content at harvest. Further, at 60d, most of the growth parameters responded maximally to concentrations lower than S_3. Treatments S_0 and S_W (controls) were at par in their effect on all parameters.

Experiment 2 (1982-83) was conducted on lentil var. T-36 during "rabi" season. The treatments consisted of pyridoxine spray at 90 or 110d of 0.0%, 0.1%, 0.2%, 0.3%, 0.4% and 0.5% and were

designated as $F_{W(90)}$, $F_{1(90)}$, $F_{2(90)}$, $F_{3(90)}$, $F_{4(90)}$, $F_{5(90)}$, $F_{W(110)}$, $F_{1(110)}$, $F_{2(110)}$, $F_{3(110)}$, $F_{4(110)}$ and $F_{5(110)}$ respectively. The effect of these spray treatments on the parameters selected in Experiment 1 was noted at 120d and at harvest. There was also an unsprayed control (F_0) in the scheme of treatments.

Treatment $F_{2(90)}$ proved optimum for almost all parameters, whereas for yield parameters $F_{2(110)}$ was equally effective. Treatment F_0, $F_{W(90)}$ and $F_{W(110)}$ showed equal effect.

Experiment 3 (1983-84) was also conducted on lentil var. T-36 during "rabi" season to study the combined effect of soaking the seeds for 12h in 0.0%, 0.2%, 0.3% and 0.4% and spray of 0.0%, 0.1%, 0.2% and 0.3% aqueous pyridoxine solution at 90d. The sixteen combinations were designated as S_W+F_W, S_W+F_1, S_W+F_2, S_W+F_3, S_2+F_W, S_2+F_1, S_2+F_2, S_2+F_3, S_3+F_W, S_3+F_1, S_3+F_2, S_3+F_3, S_4+F_W, S_4+F_1, S_4+F_2, S_4+F_3. The parameters were the same as in Experiments 1 and 2. Of these, growth parameters, NRA and leaf NPK content were studied at 60, 90 and 120d; NAR was determined for 60-90d and 90-120d intervals and yield parameters and seed protein content were noted at harvest.

Treatment S_3+F_W proved optimum for growth parameters at the later two stages (except for root length at 120d and for root nodule number at 60d that were optimum in S_W+F_3 and S_3+F_W respectively); NAR during both intervals; NRA and leaf NPK content

at all three stages and all yield parameters (except 1,000 seed weight that was non-significant) and seed protein content at harvest.

Experiment 4 (1983) was performed on summer moong (Vigna radiata L. Wilczek) var. K-851 during "zaid" (summer) season to investigate the effect of pre-sowing seed treatment for 4h with 0.0% (S_W), 0.1% (S_1), 0.2 (S_2), 0.3% (S_3), 0.4% (S_4) and 0.5% (S_5) aqueous pyridoxine solution on growth parameters (plant length, root length, root nodule number, fresh weight and dry weight), net assimilation rate (NAR), nitrate reductase activity (NRA), leaf NPK content, yield parameters (pod number, pod length, seed number/pod, 1,000 seed weight and seed yield) and seed protein content. The growth parameters, NRA and leaf NPK content were studied at 20, 30, 40 and 50d; NAR was computed for 20-30d, 30-40d and 40-50d intervals and yield parameters and seed protein content were determined at harvest.

Treatment S_3 proved optimum for almost all growth parameters (exceptions being leaf number, fresh weight and dry weight at 20d that were non-significant), NAR, NRA and leaf NPK content and yield parameters, except 1,000 seed weight that was optimum in S_W. However, plant length (at 20d) and root length at 20d as well as seed protein content were maximum in S_1 and S_2 respectively.

Experiment 5 (1983) was conducted on summer moong var. K-851 during "zaid" season to study the effect of foliar spray at 35 or 45d of 0.0%, 0.025%, 0.05%, 0.1% and 0.2% aqueous pyridoxine solution on the same parameters as in Experiment 4. These treatments were designated as $F_{W(35)}$, $F_{1(35)}$, $F_{2(35)}$, $F_{3(35)}$, $F_{4(35)}$, $F_{W(45)}$, $F_{1(45)}$, $F_{2(45)}$, $F_{3(45)}$ and $F_{4(45)}$ respectively. Growth parameters, NRA and leaf NPK content were studied at 45 and 55d; NAR was computed for 35-45d and 45-55d intervals and yield parameters and seed protein content were determined at harvest.

Treatment $F_{3(35)}$ proved optimum for all parameters studied, except root length at 55d (non-significant) and 1,000 seed weight that was optimum in $F_{1(45)}$. Treatment $F_{3(45)}$ proved as effective as $F_{3(35)}$ for pod length and seed number/pod. Treatments $F_{W(35)}$ and $F_{W(45)}$ were at par in their effect on all parameters.

Experiment 6 (1984) was also performed on summer moong var. K-851 during "zaid" season to study the combined effect of soaking the seeds for 4h in 0.0%, 0.3% and spray at 35 or 45d of 0.0%, 0.1%, 0.2% and 0.3% aqueous pyridoxine solution in ten combinations, i.e. $S_W+F_{W(35)}$, $S+F_{W(35)}$, $S+F_{1(35)}$, $S+F_{2(35)}$, $S+F_{3(35)}$, $S_W+F_{W(45)}$, $S+F_{W(45)}$, $S+F_{1(45)}$, $S+F_{2(45)}$ and $S+F_{3(45)}$, on the same parameters as in Experiments 4 and 5. The growth parameters, NRA and leaf NPK content were studied at 45 and 55d; NAR was computed for 35-45d and 45-55d intervals and yield parameters and seed protein content were studied at harvest.

Among different treatments, $S+F_{W(35)}$ and $S+F_{W(45)}$ proved optimum for all parameters, except plant length at 45d, leaf number at both samplings and fresh weight at 45d which were maximum in $S+F_{1(35)}$, $S+F_{2(35)}$ and $S+F_{1(35)}$ respectively. Treatments $S_W+F_{W(35)}$ and $S_W+F_{W(45)}$ were at par in their effect on all parameters studied.

These results have been discussed in the light of the data of other research workers in Chapter 5.

The information contained in this thesis adds to the literature on the growth and development of crop plants in the following respects.

1. Seedling emergence seems to be the first critical period when exogenous supply of the vitamins through seed treatment becomes imperative to augment the performance of crops with low seed pyridoxine content.
2. Soaking of seeds in pyridoxine solution exhibits beneficial (albeit variable) effect on different organs throughout the entire life of the plant.
3. Various organs of the plant require different amount of pyridoxine for their optimum growth and development.
4. Flower-initiation stage (90d in lentil and 35d in summer moong) seems to be the second critical period for foliar application of pyridoxine. At this stage, competition starts between vegetative and reproductive growth for utilising the vitamin *per se*.

5. Seed-soaking as well as foliar spray treatments enhanced growth parameters, NAR, NRA, leaf NPK content, yield parameters and seed protein content of lentil and summer moong.
6. Growth characteristics, NAR, NRA, leaf NPK content and yield characteristics may be used for predicting the productivity of lentil and summer moong. Of these, root nodule number, NAR, NRA, leaf NPK content and yield parameters showed consistent strong correlation with seed yield. On similar consideration, NRA and leaf NPK content proved more reliable for predicting seed protein content.
7. The optimum (and economical) concentration of pyridoxine for seed soaking for most of the parameters, including seed yield and quality, was found to be 0.3% for both crops. However, the optimum spray treatment at flower-initiation stage for lentil was 0.2% and for summer moong, 0.1%, soaking proving superior over spray.

It is, therefore, concluded that pre-sowing seed treatment with 0.3% pyridoxine solution may be exploited to augment the yield and improve the seed quality of lentil and summer moong.

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A P P E N D I X

PREPARATION OF REAGENTS

The reagents for various biochemical determinations were prepared according to the following methods.

1. Reagents for pyridoxine estimation

a. Chloroimide reagent

100mg of crystalline 2,6-dichloroquinone chloroimide was dissolved in 250ml of isopropanol. The solution was kept in a glass-stoppered bottle in refrigerator and discarded when pink colour developed.

b. Ammonia-ammonium chloride solution

160g of ammonium chloride was dissolved in 70ml of distilled water in which 160ml of concentrated ammonia water (approximately 27%) was added. The solution was diluted upto 1l with distilled water.

c. Boric acid solution

5g of boric acid was dissolved in 100ml of distilled water.

d. Pyridoxine hydrochloride solution

100mg of pyridoxine hydrochloride was dissolved in 1l of distilled water which was kept in an amber coloured bottle in refrigerator.

e. Buffer solution (pH - 3)

73g of sodium phosphate dihydrate and 167g of citric acid were dissolved in distilled water and diluted upto 1l.

2. Reagents for nitrate reductase activity

a. Phosphate buffer (pH - 7.5)

13.6g of potassium dihydrogen orthophosphate was dissolved in 1l of distilled water (a). 17.42g of dipotassium monohydrogen orthophosphate was dissolved in 1l of distilled water (b). 160ml of solution 'a' and 840ml of solution 'b' were mixed for the preparation of the buffer.

b. Potassium nitrate solution (0.2M)

2.02g of potassium nitrate was dissolved in 100ml aqueous solution.

c. Isopropanol (5%)

5ml of isopropanol was mixed with 95ml of distilled water.

d. Chloramphenicol solution (0.5mg/ml)

50mg of chloramphenicol was dissolved in 100ml of distilled water.

e. Sulphanilamide (1%)

1g of sulphanilamide powder was dissolved in 100ml of 3N-hydrochloric acid.

f. NED HCl solution (0.02%)

20mg of NED HCl (N-1-(naphthyl)-ethylene diamine dihydrochloric acid) was dissolved in 100ml of distilled water.

3. Reagents for NPK determination

a. Nessler's reagent

3.5g of potassium iodide was dissolved in 100ml of distilled water in which 4% mercuric chloride solution was added with stirring until a slight red precipitate remained (about 325ml

of the solution was required). Thereafter, 120g of sodium hydroxide with 250ml of distilled water was added. The volume was made upto 1l with distilled water. The mixture was decanted and kept in amber coloured bottle.

b. Molybdic acid reagent (2.5%)

6.25g of ammonium molybdate was dissolved in 175ml distilled water in which 75ml of 10N-sulphuric acid was added.

c. Aminonaphthol sulphonic acid

0.5g of 1-amino-2-naphthol-4-sulphonic acid was dissolved in 195ml of 15% sodium bisulphite solution in which 5ml of 20% sodium sulphite solution was added. The solution was kept in amber coloured bottle.

4. Reagents for protein estimation

a. Reagent A

0.5% copper sulphate solution and 1% sodium sulphate solution were mixed in equal volume.

b. Reagent B (carbonate-copper sulphate solution)

50ml of 2% sodium carbonate solution was mixed with 1ml of reagent 'A'.

c. Folin's reagent

100g of sodium tungstate and 25g of sodium molybdate were dissolved in 700ml of distilled water in which 50ml of 85% phosphoric acid and 100ml of concentrated hydrochloric acid were added. The solution was reflected on a heating mantle for 10h. At the end of reflection, 150g of lithium sulphate, 50ml of distilled water and 3-4 drops of liquid bromine were added. The reflex condensor was removed and the solution was boiled for 15min to remove excess bromine, cooled and diluted upto 1l. The strength of this acidic solution was adjusted to 1N by titrating it with 1N-sodium hydroxide solution.